

Academics, cricket and apartheid

The British seem to be heading for trouble in their sporting relations with South Africa, but the case for an academic boycott remains as insubstantial as it has always been.

DURING their creative period, mostly in the nineteenth century, the British were renowned for the invention of athletic pursuits, not merely individual activities such as mountain climbing (Edward Whymper), but team games, among which cricket and rugby football are the best known. (Association football, otherwise known as soccer, which came later, is more easily understood — which probably explains why it is less distinctively a part of the snobbish British ethos.) In the course of a prosperous century, British colonial administrators, nostalgic for their childhood playing fields, took care to teach the intricate rules of their national games to those in their charge, but with the predictable result: the colonials (now all ex-colonials), being generally more athletic and, in any case, eager to make an anti-colonial argument, quickly learned how to beat the British at their own games.

That truth has been demonstrated with telling clarity this summer, when a group of Australian visitors to Britain has beaten into humiliation an English cricket team. (Cricket is not so much a British as an English sport.) For the promoters and the supporters of the game, there now remains the embarrassment of knowing what to make of the two remaining pre-arranged contests (laughingly called matches) at which the only glimmer of interest will be whether the beaten team can rise like a phoenix from the ashes of defeat. (The prospects would have been even worse if the visitors had been West Indian.) The gloom over English cricket has now been further deepened by the decision of 16 professional cricketers to spend the next two austral summers playing cricket in South Africa, the ex-colonial nation, now a free-standing republic, whose chief claim on public attention is the doctrine of *apartheid*. One consequence will be that those who go to South Africa will be excluded from the English cricket team for at least five years — prospective 'rebels' have already been dropped — so further undermining the English cricket team. Another is that there will be further endless trouble about English or, more generally, British representation in international sport. Matters are likely to be worsened if rumours that British rugby footballers are about to set off for South Africa should be confirmed.

On the face of things, this impending fuss, based on a formal agreement between sporting authorities within the British Commonwealth that there should be a boycott of sporting fixtures with South Africa, contrasts awkwardly

with the opinion of journals such as this that a boycott of cultural and academic institutions in South Africa would be intolerable (see *Nature* 327, 259; 1987). Why, it may be asked, should there be one (and a harsh) rule for those who play cricket and quite a different rule for academics? The answer, luckily, is simpler than it seems, but is necessarily dependent on knowing what is happening in South Africa.

First, the case for a boycott of South Africa in sports is strong and unchanged by the events of the past decade. South Africans play both cricket and rugby football excellently, and are forever seeking ways in which to demonstrate their skill. But those who play these games are predominantly white, and those who regulate the two sports are almost exclusively so. It is no longer against the *apartheid* rules for people of different ethnic origins to be members of the same teams, but changes in these directions have so far been largely tokens. Given the physical segregation of ethnic populations, cricket or rugby football teams formed naturally are likely to be of uniform ethnic composition. In any case, the people of the black and coloured townships have other things on their minds than cricket and rugby football. It might be different if they believed that excellence would allow them, in their turn, to beat white South Africa at its own games.

In the circumstances, the only surprise is that the international cricket authorities seem content with a five-year ban on those who play in South Africa. Why not a lifelong ban? The case of the South African universities is different. At least two of them (Witswatersrand and Cape Town) are citadels of enlightenment in an otherwise blinkered land. At great risk to their own immediate interests, they are a principal source of higher education for black and coloured populations in South Africa. But even Afrikaans universities such as Stellenbosch have recently surprised even themselves by the liberality of their opinions on social matters even if they have not yet squarely faced the need that the attainments required of entering students should be adjusted to suit their preparation at school.

Moreover, there is every reason to expect that the same institutions will have a crucial part to play in the post-apartheid society that cannot be much longer delayed in South Africa. That is why there must be one rule for academics and another for sportsmen. □



Congress reminded of perils of space flight

■ Shuttle accident statistically likely

■ Alternatives needed for flexible programme

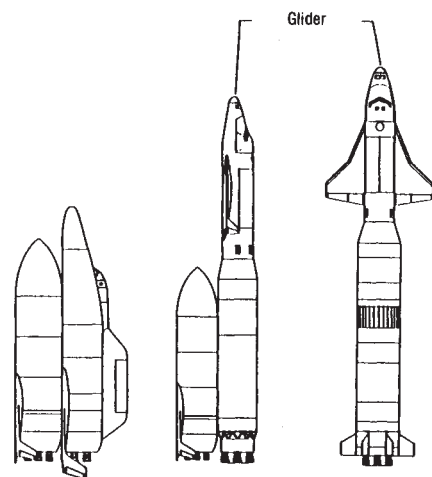
Washington

JUST two weeks after US President George Bush celebrated the twentieth anniversary of the moon landing with renewed enthusiasm for the space station and a stirring call for a manned mission to Mars, the congressional Office of Technology Assessment has issued a sombre reminder that manned space flight is still a very risky business.

The report says that it is "statistically likely" that another space shuttle will be lost or damaged if launches continue at the

a little flexibility would be for Congress to order a fifth shuttle. Three shuttles are ready and a fourth, the Endeavor, will be complete in 1992. But a fifth would cost around \$2,500 million and could not be ready until 1996, too late to be of help in building the first phase of the space station. And by that time the shuttle design will be more than 25 years old.

Flying the shuttles less often would reduce risks. That means alternative, unmanned launch vehicles would have to be developed. Shuttle-C, an unmanned



Fully reusable Partially reusable Expendable stages

planned rate and warns that "the United States . . . will have to accept the likelihood that loss of life will occur". If a devastating blow to the space programme by the loss of a shuttle is to be avoided, then Congress will have to decide soon what it needs — and what it can pay for — to transport humans into low Earth orbit.

Shuttle reliability is estimated at 98 per cent which means, the report says, that "there would be a 50–50 chance of losing an orbiter within 34 flights". As approximately 140 shuttle flights are needed to complete the first stage of the space station, there is only a one in eight chance that the space programme will advance that far without losing one of the shuttles.

But that grim estimate does not imply that the terrible disaster of the Challenger is likely to be repeated. A shuttle is more likely to be put out of commission by a mundane mishap — an accident with a crane during flight preparation for example — than by a major catastrophe.

The report lays out a shopping list of alternatives that could keep the space programme on schedule even if an accident occurs. The most obvious way to gain



Horizontal takeoff

Some of the 'Advanced Manned Launch System Concepts' under review. These hypothetical vehicles were penned by NASA's Langley Research Center based on 1992 technology — except for the horizontal takeoff craft which required more advanced airbreather/rocket technology.

cargo-carrying version of the shuttle offers a relatively quick solution. It could be ready by 1994 and be used to ferry space station components, thus substituting for the purchase of another shuttle. As Shuttle-C could carry 100,000-pound payloads into space, twice the capacity of a manned shuttle, fewer flights would be needed.

Launch capacity could also be increased by improving the existing shuttles. Several different options are available: further improving the solid-fuel rocket boosters, developing a new design, or switching to liquid-fuel rocket boosters. Liquid-fuel boosters are more powerful and considerably safer. Each booster would contain four motors, making a safe landing possible even if there were a partial engine failure during launch. But liquid-fuel boosters will cost \$3,000 million to develop and could not be ready before 1997.

Beyond these options comes the Advanced Manned Launch System, which could eventually replace the shuttle. Five concepts are under consideration (see figure) running from a fully reusable

Israel approves new horizons

Tel Aviv

ISRAEL'S Ministry of Science and Technology has approved next year's budget for the two scheduled payload projects under development for the Israel Space Agency's Ofteq (which means "horizon" in Hebrew) satellite programme.

The projects, each of which receives \$100,000, include an X-ray telescope developed by an interdisciplinary team at the Technion Israel Institute of Technology and an ultraviolet imager devised at Tel Aviv University. The Technion project has been finally approved for the first Ofteq III payload, scheduled for launch in 1992: the latter project is still awaiting final approval.

Both projects easily met the Israel Aircraft Industries' (IAI) requirements that a payload should weigh 20 kilograms or less and consume not more than 20 watts of energy.

The second Ofteq III payload, which has yet to be approved finally, is an ultra-violet imager that will observe "interplanetary dust at wavelengths that have not been studied until now, particularly in primeval galaxies", said Professor Yuval Ne'eman, chairman of the Space Agency.

The first Ofteq was launched last September; it was a 'deaf' satellite, Ne'eman said, being able to send signals to Earth but not receive any. With that launching, however, Israel became the eighth power in the world and the first in the Middle East with the potential capacity to gather intelligence on its neighbours.

Ofteq II, which is scheduled for take off early next year, will be controllable from Earth but Ne'eman insists that this function will not be used for intelligence purposes. It will probably be launched with the same solid-fuel Shavit rocket as its predecessor, a rocket that Ne'eman compared with the Chinese Long March III or the Soviet Vostek SA3.

Ne'eman said that lack of funds, not technological hurdles, was the biggest obstacle to the development of satellites and rocket guidance systems. He also stated that Israel had not encountered any resistance to its satellite programme.

Lisa Perlman

combination of a piloted orbiter and unpowered glideback booster, through combinations of a small glider and a powerful expendable rocket (similar to the ESA Hermes design), to a rocket-powered orbiter carried on a horizontal take-off air-breathing space plane (resembling the German Sänger design). But none could be ready before 2005.

Alun Anderson

Round Trip to Orbit: Human Spaceflight Alternatives, Special Report of the Office of Technology Assessment.

Europe delays BST decision

Munich

NEGOTIATIONS now under way in the European Communities (EC) over licensing the genetically engineered cow hormone bovine somatotropin (BST) could set the tone for years to come for the introduction of biotechnology products in the European market. Researchers, pharmaceutical companies, farmers and consumers are watching closely for an indication of whether BST will eventually be licensed in Europe.

Despite, or perhaps because of, the intense scrutiny, EC Agriculture Commissioner Ray MacSharry did not last week propose an 18-month moratorium on BST, as he was widely expected to do, in order to allow for further scientific study. Such a proposal could emerge from a meeting in September of the EC council of ministers.

BST, also known as bovine growth hormone, is a peptide hormone of 191 amino acids that is also found naturally in cows. Injected once or twice monthly, the hormone can increase milk production in cows from 15 to 30 per cent above normal. Monsanto, Eli Lilly and other US pharmaceutical manufacturers have invested large amounts into producing BST by genetic-engineering techniques, although the recombinant hormone has yet to be licensed in the United States and Europe. It would be the first genetically engineered product to be licensed in Europe for agricultural markets.

The BST issue has attracted attention because of the many interest groups affected. The differing views of consumers, environmentalists, farmers, industrialists, and United States and European politicians have created a thorny tangle from which EC bureaucrats are struggling to escape.

Field tests on BST have caused controversy since 1987 in West Germany, where environmentalists manned the barricades against the idea of a hormone-injected "turbo-cow". They objected to BST on the grounds that it might have harmful effects on milk and meat consumers or on the cows themselves. Most studies so far indicate no harmful effects on either people or cows, admitted consumer rights advocate Thomas Schlier in Bonn, but the long-term risks have yet to be determined.

Anti-BST sentiment in West Germany and other EC countries has shifted to economic arguments, according to Schlier. BST opponents ask why Europe should approve a substance that would only increase dairy surpluses and might put small farmers out of business. For example, the rank and file of UK farmers oppose its introduction, which they see as a threat to their jobs.

Pressure from the United States may

have contributed to the EC Commission decision not to request a moratorium in the licensing of BST. In a sharply worded letter that was not made public, US Agriculture Secretary Clayton Yuetter reminded EC of its pledge to liberalize agriculture trade made in the "Uruguay round" of international trade negotiations. In a 1986 "high-tech directive", EC promised to accelerate and reduce the cost of the introduction of biotechnology-derived products in Europe.

Yuetter wrote that a BST ban, or even a moratorium, would be seen as a non-tariff barrier to trade, and therefore subject to US trade sanctions.

Monsanto sees more menace in the issue. Gerhard Schwämmlein, a Monsanto executive based in Brussels, said that the

CHERNOBYL FALLOUT

Continuing plans for evacuation

London

THE government of the Byelorussian SSR last week announced contingency plans to evacuate up to 103,000 inhabitants of land seriously polluted by the Chernobyl disaster. The decision followed the publication of a new set of maps (the third this year), showing even more islands of elevated radioactivity in southern and

PACIFIC HIGHWAY

Loan for Brazil

São Paulo

THE Brazilian government has announced that the Inter-American Development Bank (IADB) has resumed loans to finance the completion of the highway that will connect Brazil to ports on Peru's Pacific coast. But the loan is dependent on the government undertaking environmental protection measures in the Amazon, including the creation of two new protected areas in the states of Acre and Amazonas, the demarcation of the limits of areas reserved for native peoples and increased satellite surveillance of the forest.

The bank suspended loans in 1987 because of fears that the project might cause the same environmental problems in Acre that were created in Rondonia when clearing of the forests led to colonization. Thousands of migrants barely subsist on the infertile soils.

Construction of the 504-kilometre highway began 30 years ago with the aim of facilitating exports from the Amazon area to Asia via the Pacific ocean. Two hundred kilometres have yet to be paved.

The government has also accepted a loan of \$8 million from the World Bank to monitor forest burning.

Ricardo Bonalume Neto

"next trade war" between the United States and the EC "could be in the offing" over BST, especially if the United States licenses BST while Europe bans it. Schwämmlein says a moratorium makes "little difference either way", as a BST "technical review" could not be completed before November 1990. But the apparent EC reversal on the marketing of biotechnology products is "depressing" enough, he said, to make the international pharmaceutical industry, and other high-technology industries, consider pulling research and development out of Europe.

Despite the harsh words, all sides seem eager to avoid repeating the mistakes of the last US-EC 'hormone war'. EC refusal to accept beef treated with steroid hormones such as testosterone led earlier this year to US sanctions, still in place, of up to \$100 million on a variety of European industries.

Steven Dickman

eastern Byelorussia. Although claims by members of the Byelorussian opposition (see *Nature* 340, 258; 27 July 1989) that the fallout-bearing cloud was 'seeded' over Byelorussia to protect Moscow have been denied by Dr Yuri Izrael, head of the Soviet meteorological service, there is no doubt that radiation in Byelorussia is causing increasing concern to the authorities. Repeated demands for personal dosimeters for the inhabitants of the affected area (which amounts to approximately 20 per cent of the agricultural land of the republic) have now, in theory, been satisfied.

The Academy of Sciences of the Byelorussian SSR this summer held a contest for an appropriate instrument, and production of the winning design has now been authorized, although the output by the end of the year is unlikely to be more than 300.

In the meantime, grass-roots anger at what is seen as Moscow's neglect of the situation continues to grow. According to *Cyrvonaja Zmiens*, the newspaper of the Byelorussian Komsomol (Young Communist League), local doctors consider that the official 'safe limit' of 35 rems for a nominal 70-year life-span is too high. Students from the Byelorussian State University, who were sent to harvest potatoes in polluted areas, were hastily withdrawn after pressure from their parents, a move that evoked even further protests from local inhabitants whose children have to remain in such areas all the year round. And when these children have been sent for a few weeks to a 'Young Pioneers' camp outside the contaminated zone, *Cyrvonaja Zmiens* notes, they have been subjected to psychological trauma by children from 'clean' areas who refuse to have any contact with them.

Vera Rich

Haemophiliacs to sue

Tokyo

A SMALL but growing number of Japanese haemophiliacs suffering from AIDS are planning to sue the Japanese government and pharmaceutical companies for their role in the import of the US blood products that are believed to be the prime source of AIDS infection in Japan.

At the end of last month, seven haemophiliacs filed suit in an Osaka district court claiming ¥805 million (about \$6 million) in damages from the state and five pharmaceutical companies — Green Cross Corporation, Baxter Travenol, Bayer Yakuhin, Nippon Zoki Pharmaceuticals and the Chemo-Sero-Therapeutic Research Institute. The suit, which claims that the government and companies failed to check the safety of imported blood products, is very similar to one filed by two haemophiliacs in May.

The number of AIDS cases in Japan is still small compared with Western nations but more than half of the approximately 100 AIDS patients and more than 90 per cent of the 1,000 or so identified carriers of the AIDS virus are haemophiliacs. They were infected by non-heat-treated blood products imported from the United States between 1979 and 1985 (when heat-treated products were allowed on the market in Japan). Earlier this year, the government and pharmaceutical companies established a fund of several million dollars to compensate haemophilic AIDS sufferers and their families but did not admit any responsibility for AIDS infection.

Critical to a resolution of the court case will be a determination of exactly when it became clear that non-heat-treated blood coagulants are a potential source of AIDS infection. Japanese haemophiliacs first called on the Ministry of Health and Welfare to introduce heat-treated coagulants in 1983. But government approval of heat-treated products did not come until two years later and Japan continued to import non-heat-treated blood products from the United States after their use was banned in the United States and other Western nations.

Ministry officials and some of their academic advisers argue that it was not clear in 1983 that untreated blood coagulants are a source of AIDS infection. They say they cut corners to approve heat-treated products quickly. But some representatives of haemophiliacs claim that approval was unnecessarily delayed.

Japanese haemophiliacs suffering from AIDS have hesitated to take legal action through fear of public exposure. But in May, Fumio Akase, a 52-year-old calligrapher, and another haemophilic, who remains anonymous, filed suit against Green Cross, Baxter Trevanol and the

government claiming ¥230 million in damages. Akase says he decided to reveal his name in the hope that it would draw attention to the plight of the approximately 2,000 Japanese haemophiliacs thought to carry the AIDS virus.

Akase's decision to reveal his name has

NUCLEAR WASTE

Billion dollar clean-up plan announced

Boston

THE US Department of Energy last week announced a five-year, \$19,600-million environmental clean-up plan for its contaminated nuclear-weapons production facilities. The plan is the culmination of a highly visible effort in the department over the past four months to come to grips with its environmental problems, and of numerous pledges from Energy Secretary James D. Watkins that the issue would receive higher priority.

While the details will not be available publicly until the end of this month, the plan commits the department to restoring its sites to compliance with federal environmental laws within the next 30 years. Among the department's promises are the release of the health records of workers at weapons facilities and the implementation of programmes to minimize waste generation.

Watkins says he hopes that the plan will restore public confidence in the department's ability to manage and operate its facilities safely, furthering what he called its "proper role as a protector of the environment".

The proposed plan commits a huge sum of federal money to the clean-up task, considerably greater even than the Energy Department's own \$14,000-million annual budget. But this may still not be sufficient. Overall estimates of the Energy Department's total clean-up bill run as high as \$200,000 million.

Senator John Glenn, a critic of the department's past "scattered and piecemeal" clean-up efforts, says the Energy Department is now "moving in the right direction". He plans to call on the General Accounting Office to assess the plan once it is released in full. Environmental groups have also welcomed the plan.

But James Beard, director of the Nuclear Weapons Project at the Environmental Policy Institute in Washington, is more critical, expressing concern that the programme is trying to do too much with too little money. According to Beard, twice as much money should be budgeted annually to address the problem fully.

Seth Shulman

caused him problems. In a court hearing on 28 July he complained bitterly that the hospital he was attending has refused further treatment. But his action is said to have encouraged the new group to file suit. Further suits are expected but the plaintiffs may not live to hear the final decision. In Japan, such court cases have often taken ten or twenty years to resolve.

David Swinbanks

CHINA

Another attack on Fang Lizhi

London

DR Fang Lizhi, the Chinese astrophysicist and campaigner for democracy, who took refuge in the US Embassy in Beijing following the military intervention in Tiananmen Square, has been formally dismissed from his post with China's Committee for Academic Degrees, the Chinese news agency, Xinhua, reported on 4 August.

Fang, who had been a member of the committee's "astronomy appraisal group" was dismissed, the agency said, because he was subject to an arrest warrant approved by the public prosecutor.

This is the latest in a series of attacks and ritual humiliations for Fang, who, in Chinese official eyes, is guilty of treachery by the very fact of fleeing to the embassy. Last month, he was removed from the posts he held in the Chinese Natural Dialectics Research Society. His past statements are quoted by the Chinese media out of context. Thus his remark last April that, if the authorities did not enter into dialogue with the protesting students, there would be an increase in tension is now quoted as an incitement to rebellion rather than an informed prediction.

The charge sheet against him includes "counter-revolutionary propaganda and instigation", and he was even accused two weeks ago in the *Beijing Daily* of advocating "the extinction of the Chinese race". Indeed, in the eyes of the Chinese authorities, Fang's guilt is so accepted that contact with him is now regularly cited as evidence of guilt in others. So, in an attack on the 'Federation of Autonomous Students Unions' which organized the sit-in at Tiananmen Square, the daily newspaper *Renmin Ribao* claimed that for more than a year some of its leading members had organized weekly seminars at which "leading associates of bourgeois liberalization, such as Professor Fang Lizhi" had been asked to speak. And in recording the dismissal of another "bourgeois liberal", Wen Yuankai from the Anhui Provincial Education Commission, Xinhua stressed that Wen is a professor at the Chinese University of Science and Technology, the university of which Fang was vice president until January 1987.

Vera Rich

LEPROSY VACCINE

India finally agrees to trials

New Delhi

AFTER nearly four years of vacillation, the Indian government has agreed to launch a human trial of the anti-leprosy vaccine developed by the World Health Organisation (WHO). India will be the third country, following Malawi and Venezuela, to test the vaccine.

The WHO vaccine, a combination of live BCG and heat-killed *Mycobacterium leprae* derived from armadillo, has been cleared by the drug controller and, according to Dr A.S. Paintal, director general of the Indian Council of Medical Research (ICMR), the trial, to be funded by ICMR, will start in January 1990.

The decision, reached at an ICMR expert committee meeting early in July, brings to end a long controversy that began at the time WHO proposed an Indian trial of the vaccine in 1985 (see *Nature* 317, 665; 1985). Although ICMR was keen, the proposal was voted down by a section of leprologists who claimed that a cheap, effective and safe indigenous vaccine was available and that there was no need to test the WHO vaccine (see *Nature* 328, 660; 1987).

Since then, two more candidate vaccines have emerged from Indian laboratories, prolonging the debate between those who favoured the WHO vaccine and those who favoured home-developed vaccines.

The plan is to use the BCG-*M. leprae* vaccine in phase-two trials on a few thousand healthy people to determine if there are any adverse effects. The trial will last four months. After that, the vaccine will be evaluated, along with two (or possibly three) Indian vaccines, in a single 'comparative' trial involving several thousand subjects at one location in south

India. The vaccine that gives the best results, whether Indian or foreign, will be recommended for India's leprosy control programme.

Paintal says the trial will be designed and conducted by a committee of experts chosen by ICMR. WHO will simply supply the vaccine and provide one scientist to sit on the monitoring committee. This arrangement constitutes a major change from earlier vaccine trials where WHO assumed total control while Indian participation was limited to provision of infrastructural facilities and manpower. By declaring it an Indian and not a WHO trial, ICMR hopes to save WHO from any possible future allegations that it manipulated the results.

The two Indian competitors in the race are the ICRC vaccine developed at the Cancer Research Institute (CRI) in Bombay and the vaccine developed at the National Institute of Immunology (NII) in New Delhi from a soil mycobacterium. A third Indian vaccine developed at the

Central Drug Research Institute in Lucknow (from *Mycobacterium habana*) may also be included in the study.

The combined trial of all the vaccines is expected to put a stop to unhealthy competition among Indian groups (see *Nature* 333, 590; 1988). "The question of which is the best vaccine for India can be found only by a comparative study", says NII director Dr G.P. Talwar. But CRI director Dr M.G. Deo still does not agree with ICMR's decision to bring in the WHO vaccine. "I have accepted participation in a true democratic fashion," he says.

Deo, who has been almost alone in campaigning against the armadillo vaccine trial, says it has none of the advantages of the ICRC vaccine developed in Bombay. He says ICMR should have restricted the comparative trials to Indian vaccines instead of bringing in the WHO vaccine which he criticizes on at least four counts: it has to be imported, requires multiple injections, is unsafe for children below 12 and is too expensive (Rs320 per dose against Rs1 for ICRC vaccine) for India, whose *per capita* expenditure on health is Rs43.

K. S. Jayaraman

WOMEN IN SCIENCE

Still a soft female touch for doctorates

Washington

THE number of US science doctorates awarded to woman has increased rapidly over the past decade, from 2,762 in 1978 (23 per cent of the total) to 3,936 in 1988 (36 per cent of the total), according to an annual survey commissioned by the Science and Engineering Education Sector Studies Group at the National Science Foundation.

But the overall rise is not distributed evenly among the sciences, as can be seen in the pair of graphs above. Women are doing better in the traditionally feminine 'softer' areas of science and worse in the pure sciences. Progress towards equality of the sexes has been slowest in mathematics; if things continue as they are, women will have to struggle for another 2,281 years before they receive as many doctorates in mathematics each year as men do now.

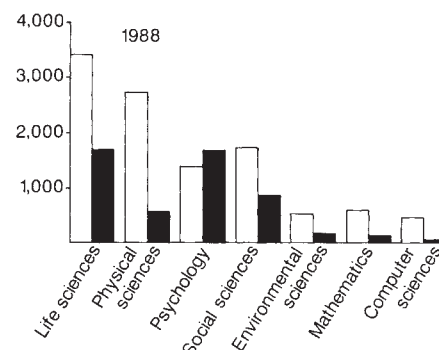
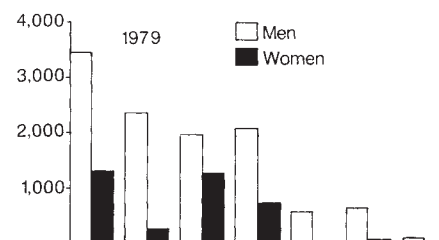
It is a different picture in the human-centred sciences. Women already receive the majority of psychology doctorates, a position they won in 1986. They were victorious in anthropology (included within the social sciences) even earlier, becoming the majority in 1984. Last year, they took the lead in sociology (also included within the social sciences).

Overall, there has been steady change in both the life sciences, where women now take 33 per cent of the total number of doctorates compared to 27 per cent a decade ago, and in the physical sciences with a change from 11 per cent to 17 per cent.

But these overall gains conceal a few

relentlessly masculine strongholds, such as physics, where only 130 out of 1,302 doctorates went to women in 1988.

The situation is even worse in engineering (not shown in the graphs). In 1988, just



under seven per cent of engineering doctorates went to women.

Women have yet to reach positions higher up the hierarchy in significant numbers. Only 3 per cent of the members of the National Academy of Sciences are women.

Alun Anderson

ENVIRONMENT

Greener Framework

Paris

AT A meeting on 27 July, the European Commission agreed a final version of its proposals for the next Framework Programme for 1990-94. With a planned budget of 7,700 million ECU (to which will be added any carry-over from the present programme), the Commission has given a green tinge to its six principal lines of action. Apart from participation in the global change programme, the Commission proposes to support remote-sensing technology and the development of an 'environment-friendly' motor car. The proposals now have to be approved by the Council of Ministers in late September and by the European Parliament. The final programme should be adopted by the end of the year.

Peter Coles

Abbott and Hybritech settle dispute

Washington

THE long-running patent dispute between Abbott Laboratories and the Hybritech subsidiary of Eli Lilly over the 'sandwich' immunoassay technique has come to an end. The two companies have entered into a confidential settlement involving "licenses and cross-licenses" of each other's sandwich-assay patents and related patent applications. The sandwich-assay technology uses two monoclonal antibodies to capture small quantities of the antigen to be detected, making an antibody-antigen-antibody 'sandwich'. The worldwide market for all immunodiagnosics is estimated to be \$5,000 million.

Abbott was banned from selling kits using the sandwich-assay technique when Hybritech brought suit against the company two years ago. The same year, Hybritech used its patent to drive Monoclonal Antibodies, Inc. out of the market, and nearly out of business, by forcing the company's marketing partner, Ortho Diagnostics, to suspend sales of several kits using the assay.

But the settlement of the Hybritech-Abbott suit leaves unanswered which company deserves the credit for first using monoclonal antibodies in immunoassays, after they had been invented by Kohler and Milstein in 1975. Some argue that using monoclonals in immunoassays should not be patentable in itself, because monoclonals offered obvious advantages over the polyclonal antibodies that were already in wide use (see *Nature* 340, 256; 1989).

The US patent office is attempting to settle the question. An investigation, termed an 'interference' proceeding, is under way to determine whether Hybritech, Hoffman LaRoche or La Jolla Cancer Research Foundation of California thought of the idea first. The three companies claim conception dates within months of one another.

Carol Ezzell

New battle over TPA

Washington

ARMED with a new patent that was issued last week covering the process of making genetically engineered tissue plasminogen activator (TPA), Genentech has filed another suit against Wellcome's US drugs company Burroughs Wellcome, its partner Genetics Institute and all of their joint ventures. Genentech is already suing all of them for infringing its patents for purified TPA and for materials used in making recombinant TPA.

The new patent will allow Genentech to protect its US market against TPA manufactured abroad and imported into the United States. The US Congress voted last year to close a loophole in US law that allowed the importing of products made abroad using US patented processes.

Carol Ezzell

Harvard fights first battle

Boston

HARVARD University, along with the firm US Biochemical Corporation of Cleveland, Ohio, filed a patent infringement suit last week claiming that the Swedish-based company Pharmacia AB has violated the school's exclusive claim to a DNA polymerase-based sequencing technology. It is the first time that Harvard has been involved in such legal action since it began patenting its scientists' work a decade ago.

The patent involves a technique developed at Harvard Medical School by researchers Charles Richardson and Stanley Tabor that uses T7 DNA polymerases to sequence DNA. Exclusive licence to the technology has been granted by Harvard to US Biochemical, which markets its T7 DNA-sequencing products under the trade name of Sequenase. US Biochemical and Harvard are seeking to stop Pharmacia and three US subsidiaries from using the technology in its own DNA-sequencing kits and other related products, and are claiming damages for past infringements which, they say, have gone on "in deliberate defiance" of and "with full knowledge of the facts" of the licensing agreement.

S. Leslie Misrock, senior partner of Pennie & Edmonds, the law firm representing US Biochemical and Harvard University in the suit, says that the patent involved is currently Harvard's "single most important patent" from a financial standpoint. Although neither Harvard nor US Biochemical will disclose how

much the university receives for rights to the technology, it is known that Harvard earns about \$1.2 million annually on about 75 biomedical patents. In addition, Misrock claims that Sequenase is a "major" product line for US Biochemical.

Misrock says the decision by Harvard to file suit was made following "very careful consideration" by university personnel and that it was regarded by them as important to "send a message that the university intends to support its licencees". Misrock also stated that US Biochemical and Harvard have identified three potential infringements on the licensing arrangement, but have decided in the Pharmacia suit to focus only on what they perceive to be "the most significant" instance.

Jorgen Winroth, a vice president of the New Jersey-based Pharmacia Inc., a subsidiary of the \$1,200-million Swedish company, stresses that his company's primary product in question, a DNA-sequencing kit, is a "very small" part of the company's overall business, making up less than \$500,000 in worldwide sales.

"Nevertheless, we do respect patent rights, and do not do anything intentionally to infringe upon them", he said, adding that the company is "by no means nonchalant" about the charges.

Winroth hopes that "a happy ending for both parties" can be reached promptly. But the lawyers for US Biochemicals and Harvard say the prospect of a reconciliation "is not even on the table".

Seth Shulman

Rival claims over DNA amplification

Washington

DUPONT is challenging Cetus Corporation's stranglehold on the lucrative market for the DNA amplification technique named the polymerase chain reaction (PCR). Last week, DuPont's attorneys asked a judge to rule that Cetus's PCR patents are invalid, but they will not disclose the basis for their argument.

Cetus received two key patents covering PCR just over two years ago. The PCR technique involves using two specific DNA probes that flank an unknown region of DNA to be studied. Through alternating cycles of heat and cooling, the probes squeeze between the strands of the unknown DNA and initiate an exponential copying process that can yield millions of copies of the unknown DNA sequence (see *Nature* 331, 461; 1988).

PCR has become widely used, and hundreds of research papers using the technique have been published in the past two years.

PCR has diagnostic as well as research

applications: it has been used to screen fetuses for genetic disorders, and several companies are developing tests using PCR to detect genetic material from the AIDS virus. The market for diagnostic PCR kits could reach \$1,500 million by 1998.

In a joint venture with Perkin-Elmer, Cetus sells a thermal cycling instrument to automate the PCR process. The cycler comes with two PCR reagent kits and costs \$8,500.

Permission to use PCR in basic research is included in the cost of the cycler and reagents. But Cetus has licensed all diagnostic uses of PCR to Hoffman LaRoche, and has said it will prosecute those who use PCR without buying one of its machines.

DuPont is also developing 'nucleic acid replication kits' and a thermocycler, and plans to begin selling them later this year.

Now that its products are approaching the market, DuPont's petition says the Cetus patents are "ripe for adjudication".

Carol Ezzell

MILITARY RESEARCH

Military cutbacks to hit MIT

Boston

UNIVERSITIES and research institutes that have come to rely on Department of Defense funds to support research look set to have to rethink their plans and prepare for retrenchment. According to Kenneth A. Smith, vice president for research at the Massachusetts Institute of Technology (MIT), the "promise of *glasnost*" and continuing federal budget constraints mean that significant cutbacks in military funds can be expected for the 1991 fiscal year.

Earlier this year a report from the Congressional Office of Technology Assessment suggested that a predicted slowing down of military spending will reverse the trends of the Reagan era. In the 1980s, research and development funding for the Department of Defense grew even faster than the overall defence budget itself, nearly doubling in constant dollars to more than \$37,000 million.

Military research cutbacks may be good for the country, said Smith, but in the short term they will cause "dislocation and a fair amount of pain". Last month, the Charles Stark Draper Laboratories, an independent military research centre affiliated with MIT until 1973, announced plans for a 10 per cent cutback in personnel. MIT itself is facing similar problems.

As MIT's research budget has grown, the military share has grown even faster, from 11 per cent of "on campus" funding in 1979 to almost 20 per cent — \$45 million

engineering and a member of an MIT committee that was formed to review military funding at the university, says the impact of cutbacks will be exacerbated because of the tight budgets of other funding agencies. Given the problems that the Department of Energy is having with its weapons production facilities, Kirtley says there are no chances of increases

EPIDEMIOLOGY

Leukaemia increase sets puzzle

London

THE UK Committee on Medical Aspects of Radiation in the Environment (COMARE) last week published a report identifying a "small but statistically significant" increase in the incidence of childhood leukaemia near Britain's two main

EPIDEMIOLOGY

Nuclear overhaul

Washington

IN an attempt to clean up the damaged image of the US Department of Energy's epidemiological research, the Secretary of Energy, James Watkins, announced last week a complete overhaul of the research programme under the supervision of an independent advisory committee.

Watkins, who assumed office in March, is reacting to criticism that research by the department has been coloured by its primary responsibility for the production of nuclear weapons. Congress is threatening to transfer the research to the Department of Health and Human Services. Watkins denies that there is a conflict of interest, but said at a hearing of the Senate Committee on Governmental Affairs last week that he was "not pleased" with the condition in which he found the programme. "It is understaffed, underfunded and under-utilized", he said, and "is buried deep within the bureaucracy."

The plan to revitalize the programme begins with the creation of a committee, whose membership will be announced this week, to advise the department on the budget and to recommend mechanisms for increasing accessibility to the department's epidemiology data, including the creation of a \$36-million data repository (see *Nature* 340, 251; 27 July 1989). As it will take at least six years to complete the repository, Watkins said he plans to make the data available now. He will announce the members of the committee this week.

Watkins has also asked the National Academy of Sciences to set up a new committee to provide the department with scientific advice on its epidemiology programme, and to control the quality of the research.

Christine McGourty

from that source. And industry will not be taking up the slack, he believes.

But Kosta Tsipis, director of the Program in Science and Technology for International Security, says that the coming reduction in military funds presents an "opportunity for matching an emerging wealth of unusual talent with manifest civilian needs". MIT, he says, should use its decades of experience in both military and civilian research to re-deploy its resources.

Seth Shulman

nuclear-weapons production facilities, the Atomic Weapons Research Establishment near Aldermaston and the Royal Ordnance Factory at Burghfield, both in Berkshire.

The committee found that 41 children were registered with leukaemia compared with an expected number of 28.6 based on the average incidence of leukaemia in England and Wales. All the excess cancers were in children under four years old.

But the committee, chaired by Professor Martin Bobrow, head of paediatrics at Guy's Medical School, said the increase could not be attributed to radioactive discharge from the installations because accidental and authorized emissions were too low. A number of possible explanations were considered, including other potential sources of radiation, but the report concludes that a combination of factors is most probably involved.

In two previous reports, the committee identified increased incidences of childhood leukaemia around the nuclear reprocessing plants at Sellafield and Dounreay. It now says that further research is needed to confirm the findings of all three reports and more information is needed on cancer rates in the rest of the country. The central recommendation is that the geographical distribution of cancer should be studied on a nationwide basis with the aid of an improved registration system. It also recommended that a sample survey of the levels of radionuclides in households around Aldermaston and Burghfield should be carried out.

Leo Kinlen, of the Cancer Research Campaign's epidemiology unit at the University of Edinburgh, believes the results are consistent with the theory that an influx of population can bring an infective agent into a local community which has not built up a resistance to it. This theory is supported, he says, by the clusters of leukaemia found in the isolated populations of Sellafield and Dounreay where the nuclear power plants generated the need for an influx of specialized workers. Although the weapons plants are not in isolated areas, the populations have increased significantly since the 1960s, says Kinlen.

Ben Webb



— today. Some research areas at the university, such as the department of aeronautics and astronautics and the artificial intelligence laboratory, receive more than three quarters of their research funds from the Department of Defense.

James Kirtley, a professor of electrical

Education a burning issue

Oxford

ON 6 September 1989, F. W. de Klerk seems destined to succeed P.W. Botha as South Africa's State President. But he faces no easy task: polls predict that in the general election to be held on that date, the ruling National Party will lose seats to both the ultra-right Conservative Party, the official opposition, and the newly formed Democratic Party to its left. The government is certain to be faced with a reduced majority, and the possibility of a hung parliament has not been ruled out.

As Minister of National Education for the past five years, de Klerk has presided over a general increase in education spending, but his insistence on racially segregated schooling has prevented this increase from being used efficiently. He abolished racial restrictions on admissions to the universities, but has failed to provide the tertiary sector with the resources to prevent an erosion of academic standards. As Minister of National Education, de Klerk has a co-ordinating and policy-making role for the country's four other education departments: those of white, coloured, Indian and African education.

Unlike his predecessor, Gerrit Viljoen (former rector of the Rand Afrikaans University), who was demoted to be Minister of (African) Training and Development Aid in a cabinet re-shuffle in 1984, de Klerk had had little experience in the field of education before assuming this post. He practised as a solicitor for 12 years before entering parliament in 1972.

Education remains a burning issue for South Africa's disenfranchised majority, as it is structural inequalities in primary and secondary education which, more than any other factor, restrict the upward mobility of black South Africans.

Education currently claims 20 per cent of all government expenditure, which is a drastic improvement on the dark days of the 1960s and 1970s, and one for which de Klerk (and Viljoen) can claim some credit. But de Klerk remains fiercely committed to the concept of racially segregated education at the primary and secondary levels.

A decline in white births in the 1960s has meant that there are now many vacancies in white schools, while recent increases in spending on education have been absorbed by building more black schools to accommodate an increasing demand for places. There have been hardly any funds left over to redress the structural inequalities in *per capita* expenditure on education between whites and blacks, which have narrowed only marginally during de Klerk's tenure.

In the tertiary sector, de Klerk's initiatives seem more enlightened. His director-general of national education, theoretical

physicist Dr Roux Venter, devised a new formula for the financing of government subsidies to the country's universities, which takes undergraduate and post graduate numbers, and their respective success rates, into account separately and weights them differentially, as well as making provision for a component based on research output as measured by articles published in reputable journals.

But the introduction of the formula in 1985 coincided with the collapse of the country's economy and consequently it has never been implemented properly. For the first three years, the universities received their entitlement in terms of the formula minus a blanket 17 per cent. Last year, the cuts applied to individual uni-



F. W. de Klerk, next state president.

versities varied considerably, and mysteriously, but do not appear to have been politically motivated. This year's subsidy allocations saw the virtual collapse of the formula, but with the acquiescence of the universities themselves.

A meeting of the Committee of University Principals (CUP) was presented with a scenario of the proper implementation of the formula for 1989: the entitlement for this year divided by the achievement last year for each university would have resulted in an award of between 5 per cent more and 5 per cent less than the sum awarded last year in all but four cases. The four were the homeland universities of Fort Hare and Zululand, which should have received 45 per cent and 30 per cent less respectively than they did last year, and the universities of Cape Town (UCT) and the Western Cape (UWC), which should respectively have received 18 per cent and 46 per cent more. The CUP recommended that as a matter of priority, no university's cut should be more than 5

per cent of last year's award, but requested a further R25 million to make the increases due to UCT and UWC, while simultaneously bailing out Fort Hare and Zululand by restricting their subsidy reductions to 5 per cent.

Needless to say, the government was unable to find the funds to make the increases due to the two universities it perceives as the most subversive. Both universities had experienced a drop in enrolment in 1986, chiefly because of school boycotts in the Western Cape the previous year, which had resulted in fewer students matriculating in their major area of catchment. Subsidy entitlements are calculated on the basis of enrolment and performance figures of the year two years before the award is made, so both had relatively low entitlements last year, but far larger ones this year as a result of substantial growth in 1987.

Whether de Klerk lacked the clout to extract a further R25 million from Finance Minister Barend du Plessis (whom he narrowly pipped to the post in the National Party's leadership contest in March), or whether he simply lacked the will, remains open to speculation.

Despite an increase of 23 per cent earlier this year, the inflation-adjusted income of academics has declined by some 30 per cent (lecturers), 35 per cent (senior lecturers) and 37 per cent (professors) since 1971. In the higher ranks, fiscal drag has led to net incomes being even lower. De Klerk's response to this has been to wash his hands of the issue by declaring the universities responsible for setting their own salary scales (within certain limits), a measure which, in the context of not implementing the subsidy fully, amounts to passing the buck to the university councils.

By contrast, the high-handed Health Minister Willie van Niekerk has proved to be a remarkably effective lobbyist in ensuring a continued high level of remuneration for the country's medical fraternity, a factor which in turn has caused much dissatisfaction within the universities, which are obliged to pay medical academics with clinical duties on the same scale as their counterparts in the health service.

De Klerk's major confrontation with the universities has related not to his apparent inability to secure enough funding to prevent the erosion of standards in tertiary education, but to his attempt to impose politically motivated subsidy conditions on the universities in August 1987. The universities of Natal, Cape Town and the Western Cape were successful in having these conditions declared null and void by the Supreme Court. De Klerk agreed to respect the court's decision, leading to speculation that he himself had been unenthusiastic about the conditions in the first place, but had been

ALASKAN OIL SPILL

obliged to implement a cabinet decision to impose them.

His term of office must be credited with one major reform: the abolition, in 1986, of the remaining restrictions on the autonomy of the universities to decide who they could admit as students. (Racial criteria on admissions were imposed by the government in 1959, and remained in place in some fields of study until 1986).

As a consequence, black students now comprise between 19 per cent and 28 per cent of the student body at the English-speaking campuses, whereas in the country as a whole, 84 per cent of the population but only 40 per cent of university students are black. Black students comprise 9 per cent of the student body at the dual-language University of Port Elizabeth. At the Afrikaans universities, these figures are lower, ranging from 5 per cent at Stellenbosch to only 0.3 per cent at Pretoria.

The universities at present claim 15 per cent of the education budget. While not ruling out the possibility of a modest increase in this figure, de Klerk has prescribed rationalization as the key to solving their financial problems, and it is in this context that he abolished statutory racial admissions criteria. Yet he has held back from forcing rationalization upon the universities, stating that he would rather it were self-imposed.

An obvious possibility would be to downgrade the status of universities with few postgraduate students to that of American-style colleges. This is a measure unlikely to be willingly self-imposed by these institutions and one which would be politically sensitive: most of the likely candidates are homeland universities set up for each ethnic group as part of the masterplan of grand apartheid. As he lacks the political courage to implement the logical consequences of rationalization himself, de Klerk has resorted to squeezing the universities financially in the hope that they will implement it themselves.

But de Klerk's brand of conservative pragmatism, in the context of South African politics, is not the worst possible prospect.

He has at least displayed that he has some respect for constitutional government, a notion that appears almost entirely alien to President Botha. In addition, he has a renowned distrust of the "securocrats" who surround the state president, and into whose hands much of the country's administration has passed in recent years. If technocrats such as Roux Venter are to replace the major-generals as the mandarins of the new regime, there may be cause for cautious optimism. Either a reduced majority for the National Party in September, or a decline in his own support within the party, could cause de Klerk to bend his pragmatism in a more enlightened direction.

Michael Cherry

Survivors return to the wild

San Francisco

IN the first major attempt to determine the effect of the Alaskan oil spill on marine mammals, 28 sea otters tagged with miniature radio-transmitters were released back into Prince William Sound late last month. The otters had been rescued from the oil-polluted sound after the Exxon tanker *Valdez* struck a reef in March, spilling 10 million gallons of crude oil into the water.

Biologists at the US Fish and Wildlife Service (USFWS) hope to learn whether the otters can return to the waters where they were rescued and whether they can survive in the wild after being in captivity for as long as four months. The answers will help the service to decide what to do with the 170 sea otters still awaiting release. Over the longer term, the study will assess whether food sources have been contaminated and will compare the survival rate of the released mammals to that of other radio-tagged otters that remained in the wild throughout the spill.

Most of the otters released last week carried surgically implanted radio-transmitters designed to broadcast signals for 30 months. The otters — 14 females and 14 males — were set free in clean areas of the sound's eastern section. Much of the crude oil has moved out of the sound or congealed into easily avoidable clumps. "I don't think there have been any new mortalities recently", said Tony DeGange, a wildlife biologist from the US Fish and Wildlife Service (USFWS) in Anchorage. "I wouldn't say it's over, but certainly the oiling from heavy oil is over." According to USFWS figures released on 3 August, 28,734 birds, 905 otters and 122 bald eagles died as a result of the spill. Thirty four otters and 671 birds, including 15 eagles, have been released back into the wild after treatment to remove oil.

Scientists are now hurrying to gather the data needed to assess the total damage

caused to the environment before winter arrives and field work becomes impossible. An effort is being made to examine any national resources that may have been affected — fish, mammals, birds and any other wildlife. No conclusions will be drawn until autumn or winter by which time researchers will have had a chance to compare new data to previous studies.

Alaska's huge fisheries industry suffered immediate damage in both Prince William Sound and around Kodiak Island to the south-west. The herring-roe harvest in the sound, which totalled \$12.2 million last year, was lost completely as officials closed the season for fear of contamination. The salmon industry suffered an even worse fate, with two of 11 salmon districts closed entirely because of heavy oil in Prince William Sound, site of a \$76-million harvest last year. Almost the entire season was lost around Kodiak Island, where salmon fishermen netted \$94.4 million in sales last year.

News of the otter release came as a leaked internal memorandum from the general manager of the clean-up operation at Exxon drew an angry response from some environmentalists and state officials. The memorandum revealed that the company planned to end clean-up operations for the winter by mid-September. Exxon employs nearly 11,000 primary and support workers in its clean-up operation, which covers vast areas of sea and about 728 miles of coastline.

Exxon was quick to explain last week that the memorandum did not mark a deviation from the clean-up plan formulated last May, but simply a gradual withdrawal before winter arrives. Exxon claims it will complete the clean-up by mid-September. Its workers have successfully treated some 600 miles of affected shoreline, often in remote and rocky areas accessible only by boat or helicopter.

Robert Buder

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

An oil-containment boom in Prince William Sound failing in its job: there is oil on the right side of the boom, which is moving towards the shore (Photo: AP).

Jason still a stealthy power

La Jolla

OPERATIONS wound down last week in a top-security compound above the affluent southern Californian town of La Jolla where an elite body of 45 scientists has met for the past two months. No-one outside the group knows exactly what has been under discussion and no conclusions will be made available to the public. Even a list of those attending is hard to obtain.

The group is known as 'Jason'. It has met every year since 1960 and exerts a powerful influence on US defence policy — yet few people even know it exists. Most of the topics it examines are classified. But many involve ideas as far in the future as Star Wars (the Strategic Defense Initiative) was 26 years ago, when the group first discussed the issue.

Almost every member of Jason is a physicist or an engineer. Their aim is to set aside politics and independently evaluate advanced defence projects and new concepts. Jason has included at least eight Nobel prizewinners, among them Charles Townes of the University of California, Berkeley, Murray Gell-Mann of the California Institute of Technology, and Joshua Lederberg, president of Rockefeller University.

Jason began with Project 137, set up in 1958 to involve academic people in defence and national policy decisions. It ended after only one year but the idea lived on in a new group organized by distinguished scientists, including Townes, fellow Nobel laureates Hans Bethe and Eugene Wigner, and black-hole pioneer John Archibald Wheeler. Over the years, they attracted a mix of older talent and up and coming stars. The name Jason was chosen because members felt the search for the Golden Fleece captured the mood of the venture.

The Jasons gather from mid-June until early August in La Jolla on the grounds of GA Technologies. Their headquarters, in Building 29, is surrounded by trees and ivy and is swept regularly for electronic eavesdropping equipment. Separate clearances are needed to enter both it and GA Technologies.

Although no-one knows exactly what they discuss, one of this summer's major topics is acknowledged to be an examination of the 'brilliant pebbles' concept for the Strategic Defense Initiative (SDI) Office. This scheme involves placing thousands of autonomous homing missiles in orbit. A Jason team headed by John M. Cornwall, a theoretical physicist at the University of California, Los Angeles, is evaluating the idea and asking if the project is possible and whether missiles can be prevented from shooting down the wrong targets. The results of the study are confidential.

Military matters are not the only things that Jason considers. Some topics are quasi-military, such as atmospheric turbulence, which is important both in astronomy and in Star Wars laser systems. And others, focusing on environmental problems such as acid rain or carbon dioxide buildup, are not related to defence at all.

Jason has spawned many government projects. Most are classified, but one involves a method of very-low-frequency communication with deep-sea submarines. And another, created by the late Nobel laureate Luis Alvarez, centres on new ways to detect explosives at airports. The idea is being pursued by the Federal Aviation Administration and was discussed at this year's meeting.

The group's annual budget of approximately \$3 million comes mainly from the Department of Defense. But sponsors include the Defense Advanced Research Projects Agency, the Department of Energy and other, more secretive, governmental bodies. Administration and security are run through the Boston-based Mitre Corporation.

Members typically work in small groups, putting out a report at the end of the summer. The conclusions are not always what sponsors want to hear. "The thing about the Jasons is that they are, number one, an independent group and also a fairly critical group", says Dean Judd, chief scientist for the SDI Office.

Twice a year, in the autumn and spring, the Jasons gather in the Washington, DC, area for three days of technical and intelligence briefings and a chance to determine which topics they will consider in the coming summer.

Jasons rarely make statements to the press. But, off the record, they agree about the creative atmosphere inside Building 29 and what a welcome break it is from the competitive academic world. "A critical mass", is how one member describes the group. "The rate of ideas and useful information goes up exponentially. When I reach a dead end, I know who the expert on Jason is."

But Jason members also face criticism from opponents of academic involvement in military issues. Members have had their homes picketed by anti-military demonstrators. A consultancy fee of around \$500 a day helps to provide some compensation. But current Jason chairman Will Happer, an experimental physicist at Princeton University, says members receive a better reward by providing a non-military voice in defence matters. "There are very few ways an academic scientist has an influence on defense matters now — especially on classified matters. Jason is one of the few ways left."

Robert Buderl

African eradication plan threatened

Washington

THE Food and Agricultural Organisation (FAO) is planning a multi-million-dollar programme to eradicate the screwworm fly from Libya before it spreads throughout Africa, the Middle East and southern Europe, with "potentially disastrous consequences for livestock, wildlife and perhaps even human populations", according to FAO director-general Edouard Saouma. But the programme may be scrapped if donors do not provide the necessary resources.

Until the screwworm was found in Libya in 1988, it had not been seen outside the Western Hemisphere, where it had been the single most economically damaging pest of livestock. How the screwworm fly arrived in North Africa is not known.

The FAO will attempt to eradicate the screwworm fly by copying the successful sterile-male release programme carried out in Central and South America and in the southern United States between the 1950s and the 1970s. Over 100 million sterile male flies will be shipped each week from a Mexican breeding centre to Tripoli. The programme will last for at least two years at an annual cost of \$25 million. A pilot programme with a few million flies a week is planned to start within the next few months, but donors so far have only "expressed interest", says Rob Reichard, senior officer for animal health at the FAO.

If the screwworm spreads from Libya throughout Africa, the estimated loss of livestock would cost \$200 million a year.

Allan Showler, an entomologist at the Office of US Foreign Disaster Assistance, says the FAO is not tackling the problem quickly enough. Although the FAO has been aware of the problem for months, Showler says the United States has not yet received a request for funds. If there are more delays in implementing the programme, the screwworm problem could become a permanent one, he says.

Several hundred cases of livestock infestation and a few cases in humans have been reported within 100 kilometres of Tripoli since the screwworm fly was first identified. Screwworm larvae, which hatch from eggs laid in wounds, eat the surrounding flesh and enlarge the wound. If infected wounds are not treated, animals sicken and die. In the Americas, 20 per cent of infected animals die. Deaths of humans have also been reported. Lack of vehicles and insecticide in Libya is hindering attempts to control animal movement and to treat infected animals.

Neighbouring countries are monitoring border-crossing points in an attempt to contain the screwworm fly within Libya's borders, but Reichard says it is impossible to know yet whether these attempts will be successful.

Christine McGourty

Turmoil in Spain's universities

Michael J. Walker

The Spanish government plans to shorten the length of undergraduate degree courses. The wisdom of this move is questionable and its execution impossible without a major overhaul of the present system.

To what extent should governments set guidelines for university degrees? This is a burning question in Spain's universities, particularly in its 20-odd biological science faculties. Government officials and university vice-chancellors hope that shorter first-degree courses will bring Spain into line with its European Community partners. Against them are ranged students, university teachers and faculty deans, who see threats to both academic freedom and career prospects. Since April this year, classrooms in many faculties have remained empty. University teachers and students have organized local and national demonstrations — even hunger strikes — and government officials have convened meetings with faculty deans. The strong possibility that end-of-year examinations would be disrupted led the standing committee of Spain's vice-chancellors on 16 May to defer until November its final decision on curricular changes.

Several interconnected matters are at stake. First, long-standing infrastructure deficiencies make short, intensive, first-degree courses impractical without massive injections of money. Second, academic freedom is juxtaposed against political responsibility, while privileged interests resent public accountability. Third, how should the appropriateness of first-degree course structures be determined, and should the length of a first degree in a given subject be uniform? Fourth, there is growing international concern about the long-term effects of the fragmentation of biological knowledge as a result of undergraduate specialization. Fifth, how broad should the biological grounding of undergraduates in all biologically related disciplines be? And finally, would not technical, professional, or academic specialization be better served by intermediate-level postgraduate degrees following a shorter, broad first degree?

The proposals by the Spanish government would cut back first-degree courses from five years to four in economics, social studies and humanities, and most 'hard' sciences, although six-year engineering courses will only be cut to five. Medicine stays at six years, veterinary science at five, and pharmacy will be extended by six months from its present five years.

There is disquiet about how the proposals evolved and about their implications. Government officials originally consulted all university faculties and professional bodies about first-degree requirements and desirable 'profiles' for different

graduates and professionals. This resulted in about 50 policy documents, each representing the views of the discipline concerned. In most cases, the advice was accepted, even where course cuts were imposed. The biologists' profile, however, was slashed beyond recognition. Biologists say it will mean nothing to employers. Government officials say it denies biologists none of their goals. Biologists suspect skulduggery and are up in arms. Why?

Spanish first-degree courses comprise first and second cycles. The first is equivalent to pass or ordinary degree courses and the second to honours courses. There is also an optional undergraduate thesis offered by those who want a classified degree. Government proposals involve selectively cutting a year off the three-year first cycle. The teachers' protest is partly because first-year students lack the knowledge to assimilate several different subjects. This may well be true — at any rate, no risks are being taken with medicine, pharmacy or veterinary science, which are to keep their three-year first cycles. Biologists claim a similar need to give a grounding in a range of subjects more diverse than that of 'hard' sciences. They note European Community guidelines are for minimum standards only. Their concern echoes that of Professor Sir Richard Southwood's biology committee of Britain's University Funding Committee "... that traditional areas of biology are being increasingly neglected" (*Nature* 338, 363; 1989). Nobel prizewinner Professor Severo Ochoa has made the same complaint, and so has the *Colegio Oficial de Biólogos*. The view is backed by Professor James Kavanagh on behalf of the Council of the European Communities' Biologists Association, which he chairs, and which "... because of its interest in maintaining equivalence of standards for the recognition of Spanish professional biologists in other EC countries, supports the recommendation of ... studies over a 5-year period".

Students feel four-year graduates may be at a disadvantage against biologically related professionals with five- or six-year degrees, in seeking employment against a background of the highest unemployment rate in the European Community. Specialist, second-cycle-only degrees are proposed in food technology, biochemistry, and environmental and marine studies. Biologists hope to enter them after a broad first-cycle grounding, as they said in their rejected profile, but biology students

with only a two-year grounding may not meet prerequisites if, as they fear, vested interests in other faculties claim the teaching of the new degrees. Veterinary science and pharmacy could be offered food technology; chemistry could be offered biochemistry; agronomists and highway and forestry engineers could be offered environmental and marine studies. Some of these faculties already offer their own undergraduates some relevant course units and so their professional graduates might take a second degree in as little as a year by reusing credits already acquired. They could then outbid biologists seeking employment. Biology students feel outmanoeuvred and are, literally, striking back. Some vice-chancellors have expressed the view that second-cycle-only specialist degrees might be better transformed into postgraduate, 'masters', courses, on which steep matriculation fees can be levied, unlike first degrees; students and many teachers reject such attempts to privatize public universities.

Spanish students in all faculties facing cuts worry lest employers apply the rule-of-thumb that five-year graduates from one university will necessarily know more than four-year graduates from another. Equating quantity with quality would be symptomatic of underlying rigidities, both within universities and outside them. Notwithstanding guarantees to university autonomy in Spain's 1985 University Reform Law, diversity in degree structures has been whittled away by extrinsic and intrinsic factors. There is an urgent need for more and better libraries, laboratories and clerical back-up.

Professional teaching requires more tenured lecturers. Professors and lecturers have to undertake unnecessary clerical and administrative duties, leaving teaching to the untenured staff. Few untenured staff now apply for tenured appointments outside their home ground. Whereas budding lecturers used to study for national selection examinations, even though success might consign them to the other end of Spain, under democracy regionalism flourishes, with the subliminal message 'only local people need apply'.

Major overhauls of the present system are needed before short, intensive first degrees can become a practical possibility — always assuming they are educationally desirable.

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Animal experiments

SIR—Even if the death penalty for assassins diminished the risk of innocents being murdered, we should accept the risk and choose not to kill humans deliberately.

Even if scientific animal experimentation reduces the risk of human illnesses, we should accept the risk and choose not to experiment on animals.

A. TARANTOLA

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SIR—Your leading article (*Nature* 339, 32; 1989), arising from the contention that *Nature* should not have published an account of an experiment that was considered to have caused an unacceptable level of suffering to laboratory animals, hinges on the responsibility of a free press. But there may be occasions within that broader precept for restraint by the press when it is judged that harm might accrue from publication, and self-restraint of the scientific press is surely called for in the interests of maintaining high scientific standards, including ethical ones. If other journals are more lax as to what they publish, that is their business (and their readers may want to have their say). Scientific probity does not turn on the question of whether one's principles are observed by others.

Professor Colin Blakemore is clearly not in favour of free speech (*Nature* 339, 414; 1989). He would silence Clive Hollands by resort to *force majeure*, asking the Home Secretary to comment on the right of a member of the Animal Procedures Committee "to pontificate in this way". The proposal is sufficiently far-fetched not to warrant comment. Its purpose is to silence those with differing views, and for this it is necessary, of course, first to discredit them. Blakemore goes on to conceive that Hollands, who has been vilified by many anti-vivisectionists for his support for the Animals (Scientific Procedures) Act 1986, would defend the animal activists' attack on the French research establishment in question.

One of the first casualties of terrorism is, it seems, clear discourse and any modicum of civility to those of differing views. One extreme brings out its opposite, and the first to get caught in the cross-fire are the moderates. For some ten years, there has been a hard-won dialogue in the middle ground on laboratory animal welfare in Britain and Hollands has been a leading figure in it. You, Sir, warn that "there is even a danger that the moderate groups that now share credit for the improved legislation . . . will be dis-

credited by the activists" (*Nature* 339, 491; 1989). I would add "and by those researchers who take extreme positions".

Nature trots out one of the old blandishments that "given anaesthesia there is hardly any pain" (surely a revelation to anyone who has undergone any considerably surgery). But *Nature* has surely done both science and laboratory animals a service in devoting space to this issue.

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SIR—The decision by Cambridge (Massachusetts) City Council to pass unanimously the first US city ordinance to protect laboratory animals, in a country lacking in such protection, is to be welcomed (*Nature* 340, 88; 1989).

Regrettably, however, there is no animal-welfarist representation on the proposed animal-care committees, so that the essential public confidence in the committees' ability to function impartially, with the best interest of animal welfare represented, has not been secured.

David Nathan, president of the research-orientated group Citizens United for Research and Education, is reported as saying that "the council was wise enough to see that individuals who are morally opposed to any animal research cannot possibly regulate its quality". Similar sentiments were expressed during the passage of the Animals (Scientific Procedures) Act, 1986 through the House of Lords in an unsuccessful amendment on the make-up of the Animal Procedures Committee. Lord Melchett, in opposing the amendment, stated: "It is a little arrogant to assume that it will not be possible for people who hold strongly different moral perspectives on the use of animals for experiments and other abuses of animals to play a constructive part in the Committee in the future".

The welfarists who serve on the statutory Animal Procedures Committee, which includes this society's consultant, Clive Hollands, are playing a full and constructive role in the work of this committee. I believe this would also be the case with humane-society representatives serving on local animal-care committees. It is therefore of great concern that certain members of the international scientific community still hold such outdated, entrenched and arrogant positions.

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1. Hansard, House of Lords Reports Vol. 469 (23), Col. 765, 17 December 1985.

Peer review

SIR—The many recent comments on anonymous peer review have not identified its basic weakness — reviewers have virtually no incentive to do an excellent job. Scientists receive promotion, tenure and status based on 'publish or perish' and 'get grants or get out' philosophies. There is no 'review right or regret it'. Journal editors receive either salary or status but anonymous reviewers receive no pay, no recognition and no blame for a poor review. No wonder there is what Robert Crichton called "complacent reviewing" (*Nature* 337, 110; 1989) which corrupts scientific literature with errors.

For peer review to ensure scientific integrity and accuracy, some reward system for referees is needed. Alternatively, peer reviewers could be replaced by 'expert reviewers', scientists who review full-time and are held to high standards.

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Poetic justice

SIR—I can imagine no more satisfying example of poetic justice than the opportunity to refute a critic of a book review by citing the book itself as an unimpeachable authority. David A. Pyke (*Nature* 339, 248; 1989) chides me for misusing a word in my review of the *Oxford English Dictionary*. I located the origin of the word 'scientist' in discussions among 'attendees' at the 1834 meeting of the British Association. Pyke says that I have confused active and passive, that the word I seek must be 'attenders', and that if 'attendee' exists at all it can only refer to "someone attended, for example a monarch or a bride, not someone attending".

Well Dr Pyke, 'attendee' does indeed exist, and I used it correctly. Consult Vol. 1 of the *OED* and you will find the definition: one who attends a meeting, conference etc. I confess that the word goes back only to 1961 and that it is, dare I utter the phrase, an Americanism. But then, we've been around for more than 200 years now and even the *OED* cites our neologisms.

By the way, and also using the *OED* as a source, the word 'attender' goes back to the fifteenth century, but has never been used to mean 'one who attends a meeting'. (Its principal meanings are 'one who gives heed' and 'one who attends or waits upon to give service'.) Thus in inventing 'attendee', we Yanks, ever the pragmatists, were meeting a linguistic need.

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Down with the Big Bang

Apart from being philosophically unacceptable, the Big Bang is an over-simple view of how the Universe began, and it is unlikely to survive the decade ahead.

By now, of course, every schoolboy knows that the quintessential point in space-time is the occasion of the Big Bang with which the Universe is supposed to have begun. That, the argument goes, is when all the matter now in the Universe was concentrated in an infinitesimal volume so that, among other things, there could have been no ambiguity about the time such as arises in the present Universe, in which the age of distant galaxies must be less than that of ours. At the Big Bang, the Universe, whatever its constituents, must have been a point-like space at a well-defined instant.

In all respects save that of convenience, this view of the origin of the Universe is thoroughly unsatisfactory. For one thing, the implication is that there was an instant at which time literally began and, so, by extension, an instant before which there was no time. That in turn implies that even if the origin of the Universe may be successfully supposed to lie in the Big Bang, the origin of the Big Bang itself is not susceptible to discussion.

It is an effect whose cause cannot be identified or even discussed. Even the notion that the present appearance of the Universe may represent but one cycle in the oscillation of a Universe whose total mass is enough to hold it together gravitationally (which seems unlikely) would not resolve the philosophical difficulty that an important issue, that of the ultimate origin of our world, cannot be discussed.

Creationists and those of similar persuasions seeking support for their opinions have ample justification in the doctrine of the Big Bang. That, they might say, is when (and how) the Universe was created. The reality of the event is accepted. The question of its cause, in the absence of time, is a matter for the imagination. Moderate creationists are no doubt content with that inference.

Luckily for the rest of us, moderate creationists' more impatient (and noisy) brethren seem more concerned to demonstrate that the whole world began just a few thousand years ago, which is why they have impaled themselves on the hook of trying to disprove the relatively recent (and terrestrial) geological record. But, in the long run, the impatient creationists will have to retreat to the Big Bang, for which reason it may be important that Donald Lynden-Bell, J. Katz and J.H. Redmount (the first and last from the University of Cambridge's Institute of

Astronomy) have just published a calculation showing that, unless the average density of the Universe is so great that the present expansionist phase will be halted, the Big Bang could not have been a single point in space-time, but must at the very least have been a line therein (*Mon. Not. R. astr. Soc.* **239**, 201; 1989).

The argument is intricate in its detail but simple in its essence: if you seek an intuitively simple description of the real Universe, you might profitably begin by insisting on the right to use intuitive (but, in reality, mistaken) notions of what space and time are like — flat and uniform respectively.

That leads you to think of embedding the real Universe in a geometrical framework with at least one more dimension. Intuitive rules of geometry correspond to Minkowski space-time, which is flat and uniform, but which has the disadvantage of allowing only for universes that are empty of matter. In the real world, which is not empty, space-time is curved in a manner determined by the large-scale distribution of matter (Mach's principle), but that Universe can be made more tractable by embedding it in a Minkowski space-time with an extra dimension.

That means that intuitive concepts of what the world is like can be applied to a discussion of how the matter-filled Universe behaves. Lynden-Bell and his associates show that their trick works well for a two-dimensional universe (a sheet of matter) embedded in three-dimensional flat space. If the density of the matter in the sheet is great enough, it will first expand as a disk, and then contract (onto the 'Big Crunch'). They go on to show that when geometrically three-dimensional universes are embedded in flat space-time with an extra dimension, it is necessary to reconstruct the beginning of the Universe by cutting a section through the space with an extra dimension. The result is not a unique point, but a line. What that implies is that the impression that the Universe began with a Big Bang would be no less (and no more) valid from elsewhere (in time as well as space) in our own Universe, but that the estimates of when the Big Bang happened would usually be discordant with each other. On a more distant galaxy, your qualitative appreciation of the course of events through successive generations of particles might be very different from that now current here.

Purists will no doubt find room to

protest that the argument as outlined here is insubstantial. By what right can one require of the real world that it should be describable in language that is intuitively simple, obeying the prejudices of flat space-time, for example? Because that is how we think. Are not Newton's laws simply a means of codifying our knowledge of how the behaviour of the real world differs from the expectations of our flat and uniform intuition? That is merely another way of drawing attention to one of the more flagrant gaps in the epistemology of our understanding of the world we live in.

Ernst Mach was Einstein's original intellectual hero because he originated the positivist view that has been the sheet-anchor of observational science for most of the past century: reality is what you can measure, and what you cannot measure does not count. This more general principle is just as valid now as it was a century ago, as can be told from its utility in the interpretation of quantum mechanics, for example. But is it possible that the time has come for a closer enquiry into what exactly is to be understood by quantities counted as observables?

In cosmology, Doppler shifts are measurable, velocities can be inferred and, given a suitable model of the Universe, so can distances. But is it possible to measure the curvature of the Universe which, in what seems to be largely a flat space like regime, is linked primarily with newtonian gravitation? Or, in particle physics, if the properties of successive generations of material objects such as atoms, nucleons and quarks should seem related to each other like the objects in a stack of Russian dolls, is there some more durable observable whose measurement would be more illuminating?

Luckily, at least where the structure of the Universe is concerned, there may not be long to wait for an answer. It is unthinkable that the launch of the Hubble Space Telescope can be long delayed, and it is exceedingly improbable that the succeeding decade will allow the persistence of present views of how the Universe is constructed. The Big Bang itself is the pinnacle of a chain of inference which provides no explanation at present for quasars and the source of the known hidden mass in the Universe. It will be a surprise if it somehow survives the Hubble telescope.

John Maddox

A profitable lesson in heresy

Peter Parham

INSTRUCTIONAL theories used to be the immunochemists' rationalization of the seemingly infinite number of antibody specificities. The unique structure of a chemically defined antigen determined the folding and specificity of the genetically invariant, but conformationally pliable, antibody. Arthur Silverstein¹ has traced their origins to before the First World War and they persisted for 50 years, finally collapsing with Haber's demonstration that antibodies of defined specificity for antigens could be completely denatured and then refolded in the absence of antigen to give the original specificity². Thereafter, instructional ideas became fossilized in immunochemical text books. On page 443 of this issue³, however, Townsend *et al.* breathe new life into instructional theory, but in the context of the interaction between T-cell receptors and major histocompatibility (MHC) molecules that is now recognized to be fundamental to the activation and developmental selection of T cells. By showing that MHC molecules fold up around the peptides they present to T-cell receptors, and that the nature of the peptide has significant consequences for the final conformation and function of the MHC molecule, Townsend *et al.* not only revive instructional heresy, but provide a basis for many unexplained observations in the biochemical literature on class I MHC molecules.

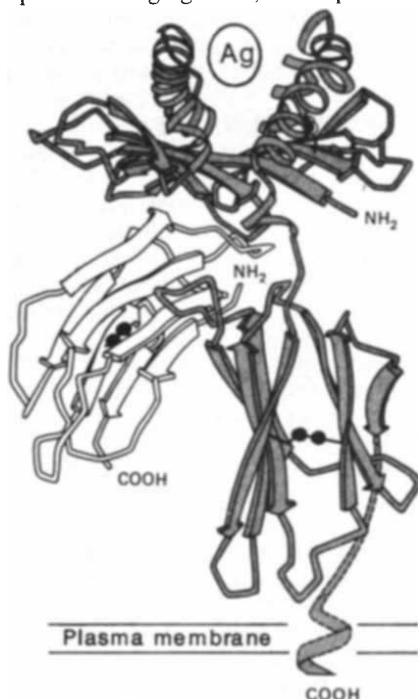
MHC structure

It has long been recognized that the association between the class I MHC heavy chain and β_2 -microglobulin (β_2 -m) is quite different from that between antibody heavy and light chains. Application of Haber's approach showed efficient regeneration of quaternary structure and antigenic activity from denatured preparations of human class I MHC molecules; but only if the denaturants were removed by simple dialysis, and not when the heavy chain and β_2 -m were chromatographically separated in denaturant, recombined and then dialysed⁴. Similar results came from translation *in vitro* of mRNA for class I heavy chain and β_2 -m: both polypeptides could be made and transferred into microsomal membranes, but no association of the two, apparently normal, polypeptides was ever found^{5,6}.

These findings suggested at least one additional component must be required for the folding of the heavy chain and its association with β_2 -m. Attempts to find such components were unsuccessful and this puzzle remained, for the best part of a decade, until it became clear that the

function of MHC molecules is to bind peptide fragments of antigens degraded inside cells (see ref. 7).

Two years ago the first three-dimensional structure revealed how peptides are bound to class I MHC molecules⁸ (see figure). The trap-like geometry of the peptide-binding groove, the presence



Structure of a class I MHC molecule, comprising a β_2 -microglobulin chain (unshaded) and a heavy chain (shaded). The latter has a carboxy-terminus that is intracellular and an extracellular antigen (Ag)-binding groove.

of presumptive peptides embedded within, and the difficulties encountered in attempts to demonstrate peptide binding *in vitro*, all conspired to suggest that bound peptide might become an integral part of the class I MHC molecule during biosynthesis. Furthermore, the peptide might be instrumental in correct folding of the heavy chain, thereby permitting association with β_2 -m^{9,10}. Townsend *et al.* now provide evidence for this general scheme and show for the first time that addition of specific binding peptides can promote folding and association of class I heavy chains with β_2 -m.

Peptide feeding experiments

Their studies focus on a mutant mouse cell line (RMA-S) that has a reduced expression (around 5 per cent) of class I MHC molecules, in this case H-2K^b and H-2D^b. The defect in RMA-S is not in the structure or expression of the genes encoding β_2 -m or the class I heavy chains, but in the association of their products. Influenza virus infection of control (RMA) cells results in

the expected binding to H-2D^b of peptides derived from the viral nucleoprotein and recognition of these complexes by specific cytotoxic T lymphocytes (CTL) with the consequent lysis of the cells. In contrast, RMA-S cells infected with influenza virus are not lysed by CTL. But both cell types are lysed if, instead of being infected with virus, they are simply incubated with a synthetic peptide containing the epitope against which the CTLs are directed. This procedure, which is commonly used by T-cell immunologists to 'sensitize' target cells to lysis by CTL without viral infection⁷, has been assumed to work through the binding of the class I molecules already present at the target cell surface. That process, however, could not explain how incubation with peptide makes the mutant cells susceptible to lysis. Now Townsend *et al.* have shown that an additional mechanism is operative, and in the case of RMA-S cells crucial, for sensitization.

Their first evidence of a second mechanism is that the peptide concentrations and incubation times required to sensitize cells to CTL lysis are greater for RMA-S cells than for RMA cells. This has been extended by the observation that peptide incubation increases the number of class I molecules at the cell surface, suggesting that the peptide may be correcting the defective intracellular association of class I MHC heavy chain with β_2 -m. In that case, the peptide must be entering the cell, and it is more appropriate to consider the cells in these experiments as being fed, rather than incubated, with peptide.

Because the peptides are entering the cell from the outside, however, it is likely that they are reaching the MHC class I molecules by a different route from that followed by peptides derived from viral proteins, such as influenza virus nucleoprotein, in infected cells. Although the intracellular pathways by which antigens are degraded and become associated with MHC molecules are poorly understood, it is generally held that class I molecules present peptides from endogenously synthesized proteins, whereas class II MHC molecules present peptides derived from exogenous proteins that have been endocytosed¹¹. In the peptide feeding experiments, the route by which the viral peptide reaches the MHC is presumably more akin to the normal route followed by peptides binding to class II molecules. The high concentration of peptide required suggests that this experimental exploitation of the exogenous pathway to allow association of peptides with class I molecules is inefficient, with only a small proportion of the ingested peptide molecules reaching the site where binding to class I can occur.

Cell-surface expression of both H-2D^b and H-2K^b on RMA-S and other cell lines can be increased by peptide feeding; thus

the effect seems to be general. On the other hand a particular peptide only increases expression of the MHC molecule to which it is known to bind. This critical result strongly indicates that the enhanced level of cell-surface expression must be due to binding of fed peptide in the peptide-binding groove of the class I molecule.

Feeding specific peptides partially, but not totally, cures the defect in RMA-S cells: cell-surface levels of class I MHC levels increase from 5 per cent to 20 per cent of RMA cells. Increased association of the H-2D^b heavy chain with β_2 -m is detected (H-2K^b has yet to be analysed), as is enhanced flux of the molecules through the Golgi to the membrane (as monitored by the sensitivity of the carbohydrate side chains to endoglycosidase H).

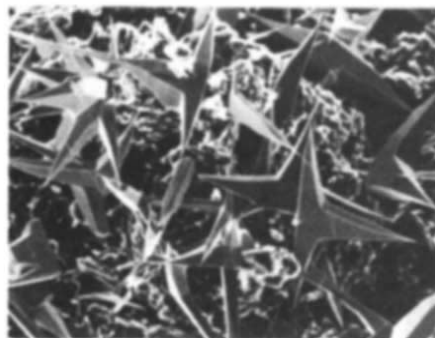
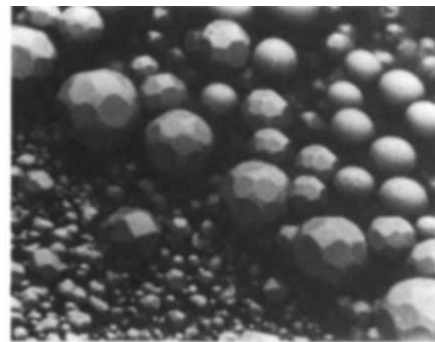
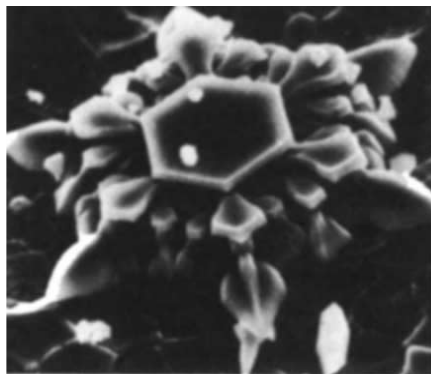
Dramatic differential effects upon β_2 -m association and exocytosis of the H-2D^b heavy chain are seen with two peptides derived from the influenza nucleoprotein. Peptide 50–63 produces much greater association with β_2 -m than peptide 365–380 (their Fig. 6a and c), yet their effects on cell-surface expression (their Fig. 6d) are comparable, suggesting that peptide 365–380 has the more potent effect.

Previous investigations of a structural mutant of H-2K^b and of the human non-classical class I molecules HLA-E and HLA-F have shown that although β_2 -m association is necessary for cell-surface expression, it is not sufficient^{12,13}. The observations with peptide 50–63 support and embellish this thesis by suggesting that differences in the peptide bound can produce conformational changes that determine whether a particular complex is exported to the cell surface, and the rate at which it happens. Thus the differential rates at which class I molecules are delivered to the cell surface may in part be attributed to the peptides they bind¹⁴. In that regard, the point mutant of H-2K^b described by Williams *et al.*¹² is of particular interest, because 80 per cent of the molecules are retained in the cell whilst 20 per cent egress in a manner that is comparable to wild type. This demonstration that peptides can prevent their own presentation by inhibiting exocytosis has implications for tolerance, autoimmunity and the evolutionary strategies adopted by pathogens to evade the immune system.

Presentation pathways

The nature of the mutated gene in the RMS-A cell remains unknown. Townsend *et al.* suggest the lesion may lie in proteins that serve to transport peptides across membranes from one cellular compartment to another. Biosynthetic studies have shown that class I heavy chains must associate with β_2 -m within about 30 minutes of translation, after which they irreversibly adopt a conformation that

Bonsai-sculptures under the microscope



Simple ternary and binary compounds are responsible for these remarkable microcrystals. Seijo Motojima and colleagues (Gifu University) use *in situ* chemical alteration of metals and chemical vapour deposition on various substrates, in some cases with electric fields, to make their microsculptures. The six-petalled ZnO flower (top left) was made from ZnO powder by Kenjiro Yamada and Shotaro Tobisawa of the National Defense Academy in Japan, using a shock wave generated by 80 grams of high explosive. □

cannot associate with β_2 -m¹⁵. The sequence of events can now be interpreted as follows. Before the heavy chain interacts with β_2 -m, it folds in a manner dependent upon interactions with a peptide: those heavy chains that fold around certain correct peptides reach a conformation that can interact with β_2 -m to form a stable complex; those that fold in the absence of peptide, or are instructed by an inappropriate peptide, reach stable but dead-end conformations that cannot bind. Intermediate situations may also exist in which a heavy chain reversibly interacts with a number of peptides before reaching a stable — either defective or functional — conformation. The correct folding of class I MHC molecules is thus critically dependent upon a supply of appropriate peptides. Townsend *et al.* postulate that for the endogenous class I pathway of antigen presentation this is provided by a transporter system that carries peptides from the cytoplasm, where they are synthesized, to the lumen of the endoplasmic reticulum where they encounter the MHC molecules. The lesion in RMA-S is

interpreted as being a defect in this transporter that would result in an increase in the ratio of defective to functional class I molecules. Peptide feeding presumably corrects the defect by providing an alternative pathway for transporting the peptides into the endoplasmic reticulum.

Various mutants with a phenotype similar to RMA-S have been described, although the reduction in cell-surface class I MHC expression and association with β_2 -m varies and is allele-dependent¹⁶. For one mutant human cell line, the genetic lesion has been mapped to the MHC although the target gene has yet to be characterized¹⁷. One possibility is that this gene is identical or closely related to that encoding the human major heat shock protein HSP70, which also maps to the HLA region¹⁸. That HSP70 molecules are frequently implicated in protein folding events directs our thoughts to alternative models in which the lesions in RMA-S and similar mutants are in a protein that controls the folding and association of the complex of peptide, β_2 -m and class I heavy chain¹⁶. Such a protein might be a

class I equivalent of the invariant chain involved in the biosynthesis and exocytosis of class II MHC molecules, which has recently been shown to contribute to antigen presentation¹⁹.

A fundamental question in immunology, raised by the MHC restriction of T cells, is how the small number of MHC molecules expressed by an individual could interact with the numerous antigens against which immune responses are made. As repeatedly found with immune mechanisms, the answer lies with multiple compounding effects. The first is to reduce the conformational complexity of protein antigens by proteolytic processing and presentation of short peptides. A second factor is the evolution of a site, formed by a defined polypeptide, that accommodates and binds a wide array of peptides with differing sequence and conformation. This was precisely the solution adopted by the instructional interpreters of antibody diversity¹. In the case of antibodies the underlying assumption that there was little sequence diversity in immunoglobulin chains proved wrong: for MHC molecules it is correct, and the findings of Townsend *et al.*, with their power to explain many previously puzzling phenomena, persuasively argue that antigenic instruction is a significant factor in T-cell responses. Thus, bound peptide is perhaps best considered as an integral and strongly associated third subunit of MHC molecules.

In this regard, the ease with which non-mutant cells can, by brief incubation with peptide, be sensitized by lysis by CTL gives a misleading impression of the stability of the cell-surface MHC-peptide complexes and the accessibility of the peptide binding groove. In such experiments it is a minute fraction of the total class I MHC molecules that take up the offered peptide and their ready detection is entirely due to the impressive sensitivity of T cells.

There can be little doubt that antigen presentation is becoming an extraordinarily rich field for protein chemists and cell biologists, as well as immunologists. Since it was first discussed in News and Views^{11,20}, much evidence for two, and probably more, presentation pathways has accumulated. Mechanisms must therefore exist for intracellular separation of the reactions whereby class I and class II molecules bind peptide. The supply of peptides to these molecules is dependent upon the pathways of unfolding and degradation for all cellular and internalized proteins, and upon intracellular transport of the peptide products. Peptides compete to instruct the folding of class I MHC heavy chains and, it now seems, to determine if, and to what extent, the resulting complex is to be routed to the cell surface. Thus, in a sense, antigen determines presentation of MHC to the T-cell receptor as MHC determines presentation of antigen. Unravelling these

pathways and mechanisms of presentation requires study both of whole cells and of *in vitro* systems. Mutant cells such as RMA-S may facilitate this analysis as they provide a system, through peptide feeding, for preferential loading of class I molecules with a single peptide, which is just what many workers in this field have long dreamed of being able to do. □

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MASS EXTINCTIONS

Manson structure implicated

Richard A.F. Grieve

DESPITE the mounting physical and chemical evidence, claims that the Earth was struck by a massive object 66 million years ago are still disputed. That such an impact caused the global mass extinction associated with the Cretaceous/Tertiary (K/T) boundary of a similar age is even more controversial¹. There are 120 terrestrial features that are the results of impacts, none of which has been convincingly linked to the K/T boundary. The lack of any corroborating evidence encourages those who dispute the impact hypothesis. But Kunk *et al.*² now claim that their radiogenic-argon dates for the Manson impact structure in Iowa show that this is the same age as the K/T boundary. The timing, however, is not everything in this debate.

Manson corresponds to an area of unusual structural disturbance discovered during the drilling of water wells. It is buried in Quaternary glacial till and is the largest known impact structure in the United States. Shocked materials have been recovered from drill holes in the uplifted central peak of Precambrian-aged granites. Kunk *et al.* report that ³⁹Ar–⁴⁰Ar dating of two samples of shocked microcline, a type of potassium feldspar common in granites, from these cores yields ages of 66.9 and 65.7 million years (Myr). They ascribe these reset ages to shock-induced heating during the formation of the Manson structure and note that these ages are indistinguishable from the K/T boundary (65–66 Myr). So is Manson the much sought smoking gun for the K/T boundary event? Kunk and others believe so. Indeed, others, with less constrained evidence on the age of Manson, have made the same suggestion³.

The problem is Manson, at 35 km in diameter, is too small to have produced the

global signature of impact and the consequences attributed to the K/T event. Thus, the age may be right but the size is wrong. The authors note that, because of the number of variables, crater scaling laws are not well known. Thus, the size (100–200 km) of the crater suggested by the much quoted K/T impact hypothesis of L. Alvarez and others⁴ cannot be estimated precisely. Although this may be the case, the problem is not so severe and the energies required to produce a crater of a given size are known to within an order of magnitude. Furthermore, other recent, well-dated craters of roughly the same size as Manson, seem not to be associated with any global biological effects (for example, Ries, 24 km, 15 Myr; Haughton, 25 km, 23 Myr; and Montagnais, 45 km, 50 Myr). Thus, the record does not support the contention that Manson-scale impacts can have global effects.

Kunk *et al.* realize this and favour an alternative interpretation, namely that Manson is responsible for the shocked quartz and feldspar grains found at K/T boundary sites in western North America and is only one of several associated impact events. This is in keeping with suggestions by some workers that multiple large impacts, as opposed to a single catastrophic impact⁴, were responsible for the mass extinction, which they consider to be a succession of smaller extinctions.

It is difficult, however, to imagine how an impact could produce species-specific extinctions on a global scale, only to be followed by other large impacts, presumably with the same basic killing mechanisms, that affect different species. Catastrophic killing mechanisms, such as the suppression of photosynthesis by global dust clouds, or shock-produced acid rain, regardless of how they are

AQUATIC VIRUSES

And now, small is plentiful

Evelyn B. Sherr

induced, have threshold values below which they do not operate. More importantly, multiple events will not have cumulative effects unless they are essentially simultaneous⁵.

Is Manson then one of a virtually simultaneous series of multiple impacts that may have occurred at the K/T boundary? Unfortunately, this is not without its own set of problems. Crater dimensions and other attributes, such as volumes of ejected dust, that might affect the biosphere, do not scale linearly with impact energy. For example, crater dimensions scale approximately as the fourth power of the energy. The impact energy required to form Manson is of the order of 10^{21} joules. A rough mass-balance calculation⁴ for the size of projectile required to account for the siderophile anomaly at the K/T boundary suggests that the energy release for the K/T event was of the order to 10^{23} joules. That is enough to form 100 Manson-sized structures or some combination of smaller and larger-sized structures. If Manson is one of several K/T boundary structures, where are the others? The terrestrial cratering record suggests that a Manson-sized structure is formed somewhere on the land surface of the Earth every 5 million years. It is possible that it is simply a coincidence that Manson has an age equivalent to the K/T boundary and that the associated impact is unrelated to the K/T extinction.

The best ages for impact events are obtained using ³⁹Ar–⁴⁰Ar dating on rocks that have been melted by the impact. These rocks have the best chance of being degassed and having their radiogenic argon clock reset. The samples analysed by Kunk *et al.* have not been melted, only shocked to much lower pressures. The argon spectra of these samples do not have a good plateau age but rather have a slight U-shape. When dealing with impact melt rocks, the minimum of the U in this type of spectrum is generally taken to represent a maximum age for the event⁶. The fact that two samples give similar ages may mean nothing more than they happen to have undergone similar shock and post-shock thermal histories, resulting in equivalent ⁴⁰Ar loss and reset ages. If impact melt rocks are discovered at Manson and also give a similar age then we can be more confident that Manson has an age equivalent to the K/T boundary. □

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ON PAGE 467 of this issue¹, Bergh *et al.* report the use of transmission electron microscopy to make direct counts of femtoplankton — viruses of less than 0.2 µm in size² in natural waters. They find up to 10^8 viruses per ml, which is three to seven orders of magnitude higher than previous abundance estimates based on plaque-forming units. The earlier estimates had led to the conjecture that instances of bacteriophage infection of suspended bacteria would be rare³; now all bets are off.

During the past few decades, aquatic ecologists have been involved in elucidating the trophic roles of ever smaller life forms (somewhat in analogy to the physicists' search for ever smaller sub-atomic particles). In the 1960s and 1970s, nanoplanktonic algae, less than 10–20 µm in size, were a major focus of investigation as the most important component of pelagic primary production⁴. In the 1970s and 1980s, picoplankton, with cells of less than 2 µm in size, were found to be much more abundant and active than previous work had suggested^{5,6}. Direct count microscopic methods revealed that the true abundance of heterotrophic bacterioplankton, 10^5 – 10^7 per ml was much greater than previous estimates of viable bacteria based on plate counts^{7,8}. Along with the greater densities of heterotrophic bacteria, the reports of high concentrations of picroalgae, including unicellular blue-greens^{9,10} and eukaryotic algae the size of large bacteria¹⁰, were electrifying.

These discoveries have contributed to a revolution in our understanding of the functioning of pelagic food webs. The original concept of a linear food chain in the sea — phytoplankton to copepods to fish — is no longer a viable model for how pelagic ecosystems operate. In fact, we are only now beginning to grapple with the idea that in many aquatic ecosystems, including much of the world ocean, most carbon fixation is carried out by cells too small for copepods to ingest — they pass right through the copepod sieving apparatus. The significance of heterotrophic bacterioplankton has received strong support from the finding that the greatest fraction of particulate organic matter in the water column of the open ocean is in fact metabolically active bacterial cells¹¹. It now appears that a complex network of trophic pathways among microbes, including algae, bacteria and protozoa, accounts for the bulk of the carbon and energy flows, with a small fraction of the total microbial production going to support all the other consumers, from copepods to whales¹².

Against this backdrop, the focus in

aquatic microbial ecology for the 1990s may well be the trophic and evolutionary implications of the high abundances of viruses in natural waters found by Bergh *et al.* As they say in their report, attack by bacteriophages could explain the unaccountably high mortality rates of bacteria measured in some systems. Viral infection can also kill eukaryotic microbes¹³, and viruses might account for some of the 'dissolved' DNA found in seawater¹⁴.

The most fundamental implication of high viral abundance is, however, that routine bacteriophage infection of aquatic bacteria is likely to result in significant exchange of genetic material¹⁵. Natural genetic engineering experiments may have been occurring in bacterial populations for eons. If bacteriophage transduction turns out to be a common mechanism for gene transfer in aquatic ecosystems, then pelagic bacterial assemblages should be able to adapt more rapidly to new situations than has been thought. They might, for example, rapidly develop resistance to antibiotics used in aquaculture, or elaborate enzymes able to act on exotic chemicals introduced into the environment. There is also the disturbing possibility that indigenous bacteria could acquire traits from pathogenic bacteria or artificially engineered bacteria released into lakes and coastal waters¹⁵.

I expect that the discovery reported by Bergh *et al.* will spur research, only just now beginning, into the genetic composition of bacterioplankton¹⁶, as well as promote further studies of the prevalence and activity of femtoplankton, the smallest 'living' particles in natural waters. □

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Pulsar puzzles for astronomers

R. N. Manchester

THE announcement in March this year by Kristian *et al.*¹ of the detection on 18 January of a pulsar in supernova 1987A with the phenomenal pulse rate of nearly 2,000 Hz stunned both observers and theorists. Almost all accepted theories of dense nuclear matter, from which pulsars are made, indicate that a pulsar spinning at this rate would rapidly disintegrate. This conclusion is borne out by calculations presented by Friedman *et al.* in *Physical Review Letters*², Shapiro *et al.* on page 451 of this issue³, and Haensel and Zdunik in a forthcoming paper⁴. But there remains considerable uncertainty about the interpretation of the observations¹, so it may be premature to abandon cherished models.

The burst of neutrinos seen with the original explosion of SN1987A strongly indicates that a neutron star was formed in the supernova, but no one expected it to be spinning so rapidly. Observations of other pulsars, especially those such as the Crab pulsar that are associated with supernova remnants, suggest that pulsars are born with relatively strong magnetic fields and pulse rates of not more than a few tens of hertz. Even for millisecond pulsars, which are believed to be very old and to have acquired their rapid spin through angular momentum transfer as a member of a binary system, the highest observed pulse rate is less than 700 Hz. The interpretation of the SN1987A pulsar as a neutron star rotating at the pulse rate puts a severe strain on models of neutron-star structure.

Stability

A stringent limit for the stability of a rotating neutron star is that its equatorial velocity be less than the orbital velocity at that radius — or material would be flung from the surface. Gravitational instabilities also set in at about this rotation rate. Because of the peculiarities of the equation of state of degenerate matter, more-massive neutron stars have smaller radii and so can spin more rapidly. But there is a limit to the mass of a neutron star beyond which it collapses to form a black hole.

Different equations of state have different mass-radius relations and different maximum masses. The new papers²⁻⁴ demonstrate that only the 'softest' equations of state (those with smallest radius for a given mass) considered so far are stable at rotation rates of 2,000 Hz and even then only for a very small mass range near the maximum. Interpretations of pulsar glitches and X-ray pulsars have favoured the stiffer equations of state. This dilemma is sharpened by the fact that the maximum mass for the soft equations of state is much less than 1.4 solar masses ($M_{\odot}$). The most accurate mass determina-

tion is for the binary pulsar PSR 1913+16 (ref. 5); both of the neutron stars in this system have masses close to $1.4 M_{\odot}$. In fact, all determinations of neutron-star masses up to the present are consistent with this value. We would not expect the neutron star in SN1987A to be much less massive.

The rapid pulse rate is not the only surprise in the detection of this pulsar. The pulse frequency varied in an approximately sinusoidal manner during the seven-hour observation. If this variation is interpreted as Doppler shift attributable to orbital motion, the companion must have a mass about the same as Jupiter ($0.001 M_{\odot}$) and its orbital radius would have placed it inside the pre-supernova star. Furthermore, despite repeated observations by the discoverers and others, the detection has not been confirmed. For the most sensitive of these observations, the limiting optical magnitude is about 21, 2–3 magnitudes (a factor of 10) fainter than the original detection. Nor have searches at radio, X-ray and γ -ray wavelengths been successful although, for various reasons, detection at these wavelengths is less likely than in the optical band.

The most probable explanation for this lack of confirmation is that the atmosphere surrounding the pulsar is only intermittently transparent. Although the outer parts of the supernova envelope have been optically thin for over a year now, we know very little about the central region. This may be much thicker and turbulent, resulting in rapid variations in the absorption and scattering along the path to the pulsar. It is difficult to understand, though, how the atmosphere could have been reasonably transparent for more than seven hours on 18 January but opaque before and since.

As is commonly the case in astronomy, there are other possible explanations for the observed phenomena that avoid the difficulties. If the object pulsed twice per spin (having a strong 'interpulse', as is observed for several short-period pulsars), the true rotation rate would be half the apparent value. But this is unlikely because Kristian *et al.* find no evidence for any signal at the sub-harmonic frequency of 984.315 Hz. More plausible, perhaps, is the suggestion made by Wang *et al.*⁶ (and others) that the pulsation is due not to the rotation of the star, but to a vibrational mode. Such modes have frequencies in the right range. Furthermore, they are likely to be damped on a timescale of the order of days by coupling with the rotation. This could explain the lack of confirmation. But the mechanism to excite the vibrations

and how these can generate pulses are not well understood, so new problems replace the old ones.

If the 2,000-Hz pulsation is due to vibration, why do we not also see the normal rotational pulsation? The most obvious reason is that we do not lie in the path swept out by the rotating beam or beams of the pulsar. For fast pulsars, the beamwidths are usually relatively wide, so that we have about a 50–50 chance of being in the beam if the rotation rate is more than 10 Hz or so. Probably more important is the effect of the rotation rate on the power of the rotating beam. Observations of other pulsars show that this is a strong function of the rotation rate and the strength of the pulsar magnetic field, especially in the optical band.

Decline

The continued decline of the supernova intensity shows that the rate of total energy loss from the pulsar is likely to be much less than that from the Crab pulsar. The exact limit is uncertain, because only a fraction of the energy loss may end up as electromagnetic radiation. Nevertheless, the indications are that either or both the rotation rate and the magnetic field strength are much less than for the Crab pulsar and hence that the pulsed luminosity is also much less. Given the distance to SN1987A, this would make the pulsar very difficult to detect.

A final explanation that must be considered is that the original detection of the 2,000-Hz pulsation was spurious. There is no doubt that the data taken by Kristian *et al.* on 18 January are strongly periodic. It is conceivable that this periodicity was generated in the equipment rather than being present in the light from the supernova. For several reasons, this seems unlikely. First, Kristian *et al.* have found no evidence for pulsations at this frequency in previous or subsequent observations with the same equipment. Second, the observed pulsations were very strong, many times stronger than the random fluctuations normally observed. Third, and most convincingly, the pulsations disappeared when the telescope was moved to another object at the conclusion of the supernova observations.

So, which ever way we look at it, the pulsar in SN1987A is a puzzle, a puzzle that is unlikely to be solved unless the pulsar is detected again. □

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Do antibodies enhance the infection of cells by HIV?

Dani P. Bolognesi

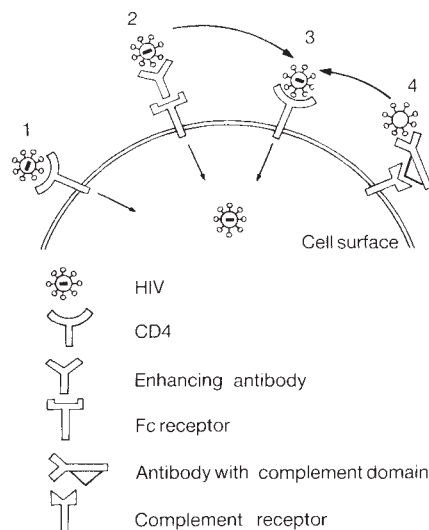
OF LATE, there has been considerable interest in mechanisms by which human immunodeficiency virus (HIV) can enter cells other than by the conventional mechanism of attachment of HIV to CD4, which serves as a cell-surface receptor for the virus. As some of the other mechanisms proposed employ antibodies or complement proteins, it is possible that immune responses to HIV would enhance rather than reduce infection of some target cells and thereby worsen the course of disease. If so, this would be bound to have a major impact on vaccination and therapeutic strategies. It is therefore worth examining the evidence, including the most recent papers^{1,2}, to assess whether such concerns are legitimate.

Antibody enhancement of viral infectivity is a phenomenon that has been recorded for alphaviruses, poxviruses, bunyaviruses, rhabdoviruses, coronaviruses, herpesviruses and reoviruses. The most poignant effect is seen with flaviviruses, such as dengue, West Nile and yellow fever viruses. In these cases, enhancing antibodies are distinct from the neutralizing antibodies that block virus infection. Neutralizing antibodies prevent fusion of the viral membrane with that of the cell within endocytic vacuoles in monocyte/macrophages and thus block entry of the virus into the cytoplasm³. In contrast, enhancing antibodies do not prevent fusion but rather facilitate uptake of the virus-antibody complex through binding of the Fc portion of the antibody molecule to Fc receptors on the target cell surface. *In vitro*, the enhancing effect of such antibodies can be as high as 10,000 fold when neutralizing antibodies are absent; *in vivo*, an excess of enhancing antibodies can exacerbate infection and the course of disease⁴.

Because primary targets of HIV infection include cells of the monocyte/macrophage lineage, which bear Fc receptors, it is logical to imagine that their infection by HIV could also be antibody-mediated. Indeed, there is some *in vitro* evidence of Fc-dependent enhancement of HIV infection^{1,5}, although there is considerable disparity in the results obtained. The confusion lies in two areas. First, can the phenomenon be routinely detected, particularly in fresh monocyte/macrophage cells? Second, is enhancement separable from the conventional CD4-dependent mechanism (1 in the figure) of entry?

As to the first issue, there is general agreement that the magnitude of the phenomenon is far less than occurs with dengue virus. When observed, it rarely

reaches more than a tenfold effect and reflects a temporary increase in the rate of infection rather than an overall level of infectivity. One difficulty in confidently observing enhancement with HIV stems from the inability to separate enhancing antibodies from other antibodies to HIV.



Conventionally, HIV infects cells after directly binding to CD4 (1). But it may infect macrophage/monocytes after antibodies mediate its attachment to Fc receptors (2) or complement receptors (4), perhaps, with subsequent transfer to CD4 (3).

Thus, the presence of enhancing antibodies in sera can only be detected once the neutralizing antibodies have been diluted out, and then only if they were present at sufficient concentrations to survive dilution themselves. Enhancement may also depend on the target cells used, the culture conditions and numerous other considerations related to the assay systems; all of which is to say that it is a measurement which is fastidious rather than routine.

The second area of confusion relates to discrepancies in the results of testing whether enhancement is blocked by agents that interfere with the CD4 receptor. Two groups have reported^{6,7} that monoclonal antibodies (OKT4A and Leu 3a) to the CD4 receptor that are known to interfere with HIV infection blocked the enhancement effect and at least one study being prepared for publication records similar results (R. Yarchoan, personal communication). By contrast, Homsy *et al.*¹ recently reached the opposite conclusion: neither OKT4A nor soluble forms of CD4 that block attachment of HIV to CD4 prevent enhancement of HIV infection of monocyte/macrophages. The

former studies suggest a mechanism whereby the enhancing antibodies serve to concentrate the virus on the macrophage surface (2 in the figure) (which, compared with the T-cell surface, is sparsely populated with CD4 receptors) and the high affinity of the virus for CD4 allows it then to transfer from antibodies to CD4 molecules and proceed to enter the cells by the normal mechanism (3 in the figure). But, if neither OKT4A nor soluble CD4 can block enhancement, it presumably involves a mechanism of entry that does not require CD4. (There is some recent evidence^{8,9} that, even in the absence of enhancing antibodies, HIV can infect cells by a CD4-independent mechanism, using an unidentified receptor.)

Fc-mediation is not the only mechanism by which antibodies can enhance HIV infectivity. Mitchell and colleagues^{2,10} have reported an effect that is mediated by certain components of human complement. Cells bearing receptors for complement would attract complement-fixing antibodies attached to HIV much like Fc receptors would bind antibody-HIV complexes (4 in the figure). This phenomenon can in some cases be studied in the presence of neutralizing antibodies provided that the titres are high enough and complement is present. Furthermore, there appears to be a requirement for CD4, suggesting that after association of the complex with the complement receptor, the virus attaches to CD4 and the normal route of infection proceeds² (3 in the figure). The most troubling aspect of these observations, however, is that the effect was only measurable in a T-cell line that is unusually rich in both complement receptors and CD4.

Whereas each mechanism can be recorded *in vitro*, evidence is still lacking that either the Fc- or complement-mediated pathways of virus entry operate *in vivo*. Most notably, neither in trials of HIV (or SIV) vaccines, nor in immunotherapy trials, is there any instance where the antibodies involved have enhanced infection by the subsequent challenge virus. (Two recent studies^{11,12} of vaccines containing killed virus even demonstrate that some degree of protection against live virus challenge can be achieved.) In some cases, high titres of antibodies that bind the virus were present whereas neutralizing antibodies were weak or absent, which approximates to the conditions needed to observe enhancement *in vitro*.

The role of enhancement in HIV infections thus requires a great deal more investigation. Experimental conditions that will allow reproducible examination of the phenomenon *in vitro* need to be identified. Identification of regions of the virus that are preferred targets for enhancing antibodies need to be defined and the antibodies purified so that they can be studied. Animal models could then be

used to evaluate more clearly the role of enhancement in various *in vivo* settings. Regardless, however, of whether or not the effects of enhancement are of major significance in HIV pathogenesis, the possibility must be eliminated that they would be induced before testing any form of vaccination or immunotherapy against HIV. □

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ASTROPHYSICS

Stellar winds and jets resolved

Lee Hartmann

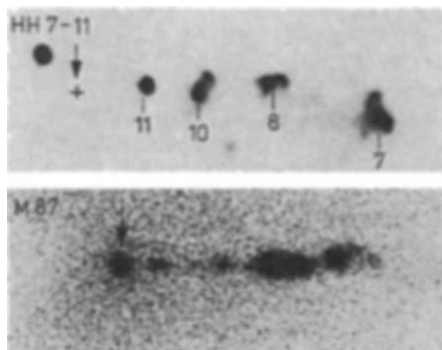
MANY, perhaps most, stars blow off large quantities of gas in early and late stages of evolution for reasons that are poorly understood. In addition to the dramatic explosions of supernovae, stellar winds are observed that are 10^6 – 10^{10} times as intense as the Sun's, and these can have profound effects on the evolution of the star and of the surrounding interstellar gas. Two new reports indicate the importance of spatially-resolved observations of stellar winds in elucidating the mechanisms of mass ejection. On page 449 of this issue¹, Moran *et al.* describe their radio-interferometric observations, using the MERLIN array run from Jodrell Bank, of an unusually active 'Wolf-Rayet' star. This is expelling material at a rate considerably greater than most stars in its class and so presents a challenge to theorists. And Reipurth recently presented² highly resolved CCD (charge-coupled device) images of a remarkable high-velocity jet — a so-called Herbig-Haro object — ejected far into space by a star that has only just formed.

Moran *et al.*¹ studied a Wolf-Rayet (WR) star, AS431, that is one of the more luminous stellar objects in our Galaxy. Such stars are remarkable for the rapid rate at which they eject material, resulting in substantial reduction in the stellar mass over the astronomically short times of 10^4 – 10^5 years³. Radiation pressure is undoubtedly responsible for the mass ejection, but present theories cannot account well for the extreme efficiency of ejection that observations of thermal radio emission indicate. Such observations can determine mass-loss rates very accurately⁴. However, some WR stars, including AS431, radiate non-thermal radio emission, the origins of which are not clear, so that mass-loss rates cannot be accurately derived for these objects⁵.

The radio-interferometric observations of Moran *et al.*, with a spatial resolution of 0.15 arcseconds, show that AS431 is a double object. The southern component, according to astrometry, corresponds to an optical star. This, Moran *et al.* argue, is

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the source of the wind radiating thermal emission. If this interpretation is correct, the derived mass-loss rate is several times the typical rate found for WR stars, making AS431 an extreme example that is particularly challenging to radiation-pressure theories. Moran *et al.* suggest a solution to the puzzle of the non-thermal



Jets represent remarkably efficient conversion of source energy into kinetic energy. The morphology, in these cases showing a series of equally spaced 'knots' of bright emission, remains similar at many scales, from stellar jets (Herbig-Haro objects), such as HH7–11 shown above and HH111 discussed by Reipurth, to galactic jets such as in M87 (below). The arrows indicate the infrared sources of the jets; the mark in the top left is a foreground star. (From ref. 7.)

emission by associating it with the second source. They speculate that high-velocity shocks (moving faster than 700 km s^{-1}) between the WR wind and the wind of a fainter companion star generate the non-thermal emission⁶.

Reipurth's report² concerns one of the very young stars known to eject substantial amounts of material in strikingly thin, high-velocity jets⁷. The jets are often contained within bipolar flows of molecular gas⁸. The flows are thought to be channelled by a dense cloud or disk around the star, left over after the star formed. The jets are far too thin to be focused by a flat disk, leading to suggestions^{9,10} that magnetic fields along the rotational axis of the system are responsible for confining

the flow. The cause of this mass ejection is not understood at present.

Reipurth studied a remarkably high-velocity jet, HH111, emanating from a young star completely shrouded from view by natal dust and gas. The jet is rendered visible by the interaction of the high-velocity ejecta with the interstellar medium, resulting in shock waves which excite optical emission lines. The emission can be traced out for a projected length of 23,000 astronomical units (1 AU is the radius of Earth's orbit), with a length-to-width ratio of about 30.

The most remarkable features of this object, however, are the bow shocks observed on either side of the infrared source. Simple continuous-flow models¹¹ for jets suggest that they should terminate in a curved bow shock, where the jet runs into the ambient interstellar medium. But in HH111, Reipurth found evidence that there are two bow shocks at each end of the bipolar ejection, as if material had been ejected in separate bursts rather than continuously.

This result is of particular interest because jets and bipolar flows often imply extremely efficient conversion of source energy into outflow kinetic energy⁸. Some pre-main-sequence objects, the FU Orionis variables, vary by one to two orders of magnitude in luminosity¹², have very large mass-ejection rates and, in some cases, exhibit jets and bipolar flows^{13,14}. Reipurth's suggestion is that intense jets arise when the rapid accretion of material from the protostellar disk¹⁵ produces short-lived bursts of energy. This model of accretion-disk-driven jets is strikingly similar to theories of extragalactic radio jets that arise from active galactic nuclei⁹ (see figure), wherein accretion onto a central black hole is the ultimate energy source. We may obtain considerable insight into the (non-relativistic) production of jets from the study of nearby young stars. □

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GTP and methionine bristles

James E. Rothman

MUCH that is of importance in cells seems to depend upon relatively non-specific (that is, energetically weak) interactions which nonetheless have to be reliable and give rise to exquisitely specific effects. For example, how do signal peptides of newly synthesized proteins effectively direct the proteins to the endoplasmic reticulum (ER) membrane when the only major common property of signal peptides seems to be overall hydrophobicity? How can errors resulting in misdirected proteins, seemingly inherent in such ill-defined signals, be avoided? Reports from the laboratories of Gilmore¹, Dobberstein² and Walter³ (the last two on pages 478 and 482 of this issue) now offer some provocative clues, raising the possibility that suitably arranged methionines act as sterically flexible detectors of hydrophobic peptides, and implying that signal decoding entails GTP hydrolysis, offering a potential kinetic proof-reading mechanism to improve the fidelity of signal recognition.

Fidelity in the face of weak interactions (such as base-pairing) is best understood for template-directed synthesis of macromolecules of defined sequence, such as in the replication of DNA and in the translation of messenger RNA into proteins. Errors in DNA replication are corrected when misincorporated residues are removed post-synthetically. As post-synthetic editing of proteins is generally not possible, earlier steps must be taken to ensure fidelity. The irreversible switch thrown by the hydrolysis of GTP is one means used to prevent misincorporation of amino acids into proteins⁴. Both codon-directed (cognate) and incorrect (non-cognate) transfer RNAs bind to ribosomes at similar rates, in a reaction mediated by a GTP-binding protein (Tu in bacteria) and followed by GTP hydrolysis, but non-cognate tRNAs dissociate much more rapidly than cognate tRNAs. The rate of GTP hydrolysis, which is essentially independent of which tRNA is bound, thus serves as an internal standard against which the persistence of the interaction of tRNA with ribosomes is timed. Only if the tRNA stays bound long enough for the GTP to be hydrolysed will a molecular 'switch' (a conformational change in the GTP-binding protein) be thrown, resulting in attachment of tRNA to the ribosome with an essentially irreversible commitment to further steps along the pathway of protein synthesis. In this way, the energy of GTP hydrolysis is used both to shift the binding equilibrium toward attachment to the ribosome and as a 'kinetic proof-reading' device, allowing each bound tRNA to be sampled before a permanent commitment is made to the

one that is most persistent and thus more likely to be correct.

An even thornier fidelity problem arose with the discovery of a spate of short and highly degenerate signal peptides that govern the distribution of newly synthesized proteins among membrane-bound compartments of cells. Unlike the signals that direct the synthesis and processing of DNA, RNA and protein, which consist of well-defined consensus sequences, most protein-sorting signals seem to be ill defined. Only some gross physical property seems to characterize each type of signal — hydrophobicity for ER targeting signals, amphipathic sequences for mitochondrial matrix signals and positively charged domains for nuclear signals. The use of a sequence-independent colligative property to direct specific interactions is a relatively new principle in biology, and may be important in other arenas such as in the distinction between 'folded' and 'unfolded' proteins made by heat shock proteins and 'chaperonins' facilitating protein assembly, and the targeting of transport vesicles for membrane fusion.

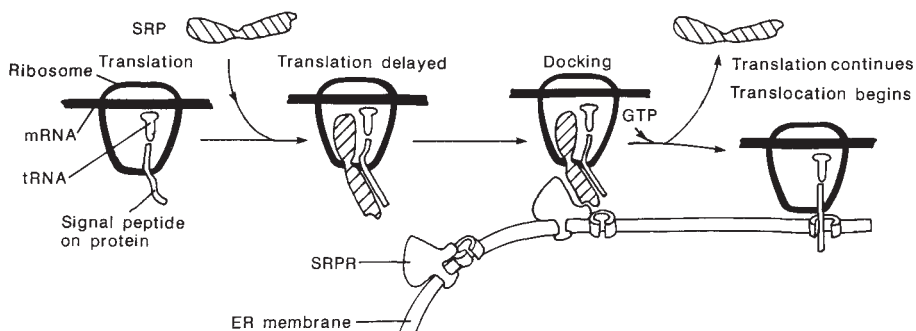
How can such weak and seemingly ill-defined signals be read and interpreted with precision? In the case of protein targeting to, and translocation across the ER membrane, which begins while translation of the protein on the ribosome is still continuing (see figure), it now seems that GTP plays an essential part in signal decoding, perhaps akin to its role in protein synthesis⁵. The signal-recognition particle (SRP)⁶, a complex of six polypeptide chains and a molecule of RNA, is in effect a dissociable subunit of the ribosome specialized for recognizing ER signal peptides. When a signal peptide is bound to its 54K subunit (SRP54), SRP delays or even arrests subsequent translation until it encounters the SRP receptor (SRPR) or 'docking protein', an integral protein found only in the ER. Then, in a complex reaction whose nature has only now been elucidated by Connolly and

Gilmore¹, the nascent chain is 'threaded' into the ER membrane translocation site, SRP is concomitantly released for later rounds, translation resumes and translocation begins.

Connolly and Gilmore show¹ that GTP binding, and in all likelihood its hydrolysis, are the key to these events. SRP-ribosome complexes bearing a 90-residue nascent chain were assembled by cell free translation. When these complexes target to the ER membrane in the absence of GTP, the ribosome-bound nascent chain still remains accessible to externally added protease, so translocation has not yet begun. Addition of GTP results in the coupled release of SRP from the ribosome-bound nascent chain and the transfer of the nascent chain into the membrane wherein it is protected from attack by protease (even when surrounding lipids are extracted with a detergent) and competent for subsequent translocation.

To what does the GTP bind? Connolly and Gilmore show that of the distinct α - and β -subunits of SRPR, only the α -subunit will bind GTP. Upon re-examining the previously published α -subunit sequence, they find that it contains a version of the tripartite GTP-binding consensus sequence found in other GTP-binding proteins such as Tu, G, and *ras* p21. Cloning and sequencing of SRP54 by Dobberstein and colleagues² and Walter and co-workers³ reveals that SRP itself also contains a GTP-binding motif, suggesting that yet another GTP molecule is involved in signal recognition and decoding. Moreover, the amino-terminal half of SRP54 (termed the G-domain by Walter and colleagues³) is homologous as a whole to the carboxy-terminal half of the α -subunit of the SRP receptor, suggesting these are evolutionarily related and may interact. Evidently, a G-domain is employed twice during signal decoding, as the SRP and its receptor interact in this pathway.

A role for GTP in SRP function is conjectural however, as there is as yet no biochemical evidence that SRP54 in fact hydrolyses or even binds GTP. This may be problematic, as signal peptide-SRP complexes apparently will form in the



Targeting of a growing protein chain to the endoplasmic reticulum (ER) membrane, mediated by signal recognition particle (SRP) and its receptor (SRPR). (Adapted from Alberts, B. *et al. Molecular Biology of the Cell* 2nd edn, Garland, New York 1989.)

absence of added GTP, and GTP-specific photolabelling of SRP54 could not be demonstrated in experiments that clearly demonstrate labelling of SRPR¹. It may simply be that SRP54 comes equipped with a pre-bound GTP as it is isolated, but this crucial point needs to be established.

This reservation notwithstanding, a number of questions about the possible role played by GTP become interesting and important. As there are now apparently at least two GTP's involved, in what order and for what purposes are they hydrolysed? Is the SRP54 GTP hydrolysed when a signal peptide is first bound to SRP as translation pauses? Is the second GTP, bound to SRPR, hydrolysed later when the SRP54-signal peptide complex is recognized by the receptor at the ER membrane? If so, as implied speculatively by Walter and colleagues³, GTP hydrolysis at both of these successive steps could be used to improve the fidelity of signal recognition. As with Tu in protein synthesis, the intrinsic GTPase rate of SRP and SRPR could serve as a pair of internal standards by which to measure the persistence of signal-peptide binding.

In one view, incorrectly matched (non-signal) peptides would have an opportunity to dissociate from SRP54 before the switch of GTP hydrolysis is thrown, fixing the bound peptide in place and stopping elongation. The bound peptide could be further scrutinized in the context of its binding site on SRP54 (just as antigen peptides are recognized in the context of MHC molecules presenting them) by the next GTP-binding protein, SRPR. Only the most persistent peptide complexes, which would contain correct signal peptides, would proceed irreversibly forward in the translocation pathway when the GTPase timer runs out and the second GTP is hydrolysed, simultaneously powering the threading of the chain and the release of SRP. Two successive rounds of such kinetic filtration would prevent almost all errors of mistaken translocation.

As intriguing as the G-domain of SRP54 is the other, carboxy-terminal half of SRP54, termed the M-domain by Walter and colleagues³ because of its remarkably high content of encoded methionine. The M-domain is without a homologue in eukaryotes, but is highly homologous^{2,3} to an *Escherichia coli* protein of unknown function predicted from an open reading frame of DNA sequence. (Walter and colleagues name this sequence *ffh*, for fifty four homologue, which is appropriate as the predicted protein would be similar in size to SRP54 and would contain both its G- and M-domains, although whether it performs a role in signal recognition in bacteria remains to be determined.)

Secondary structure algorithms applied to SRP54 and the *ffh* M-domain predict three distinct helices whose positions and boundaries coincide almost precisely in

the two proteins, and a fourth helix found only in SRP54. As the primary sequences in these regions are only 38 per cent identical, this is a striking coincidence that lends some credence to the validity of the helix assignment. It is striking and of special interest that all four predicted helices are amphipathic. Hydrophobic residues, including all of the methionine residues, in the helical regions would be clustered exclusively on one face of each helix; patches of strongly polar residues would be found on the opposite face. Three of the four helices predicted for the M-domain of SRP54 have hydrophobic faces composed mainly of methionine; the fourth amphipathic helix would be methionine-free.

This suggested organization raises the intriguing possibility that the hydrophobic ER signal peptides are bound in a pocket lined by the hydrophobic faces of the putative amphipathic helices. With this in mind, Walter and colleagues propose an ingenious, but even more speculative, solution to the enigma of how signal peptides of diverse sequences and shapes can all be accommodated within the same binding site of SRP54. They point out that methionine is unique among the hydrophobic amino acids in that its side chain is unbranched; others (including leucine and isoleucine, which are similarly hydrophobic and frequently found to replace methionine in phylogenetic comparisons) have branched side chains with sterically constrained conformations. The more flexible methionine side chains are envisaged to project into the signal-peptide-binding groove like the flexible bristles of a brush, providing a plastic environment that can accommodate itself to a variety of different (though hydrophobic) primary sequences so as to maximize close packing.

Although attractive, such notions are highly speculative. The location of the signal-peptide-binding site within SRP54 has not even been determined (in fact, Dobberstein and colleagues² suggest that peptide binding may occur within the G-domain by analogy with the location of the effector region in Tu and *ras* proteins). Nonetheless, the seminal concept of a plastic environment that detects a predominant physical property is precisely what is needed to explain the kind of signal recognition that typically occurs in protein sorting. It may well be that methionine bristles will be added to zinc fingers and leucine zippers in the growing armoury of prostheses now employed in biology. □

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Base cunning

THE roads of Britain are crumbling under the impact of the ever-heavier lorries permitted by European standards. Their relentless pounding overloads the road surface; ultimately it cracks, water gets in, and the surface breaks up. Even the best foundations yield to shock and pressure; every structure laid on the earth settles as the ground gradually compacts beneath it.

Now Daedalus has the answer. He points out that if you dig a hole, the excavated earth never fits neatly back into it. Digging has broken it up, and the resulting mass of loose and ill-fitting particles inevitably takes up more space than the original monolithic solid. Furthermore, the technology of bad concrete has made rapid strides in recent years. Additives like calcium chloride are known to embrittle it; whereas metallic iron in concrete can rust, expand and craze it. So DREADCO's engineers are devising the ultimate in bad and brittle concrete, loaded with salts, iron filings and so on. With this cheap and deplorable material as its foundation, even the most traffic-battered road should stay smooth and level. For instead of withstanding the vibration, the new road foundation will rapidly fragment. As a result, says Daedalus, it will use the force of the traffic against itself in a sort of road-maintenance Zen.

The surface of the road will be the usual slightly flexible, tarmac-topped structure. It transmits traffic vibration down to the new foundation concrete, which soon breaks up into a mass of small, close-fitting particles, rather like a shattered windscreen. Continuing traffic vibration shifts and tumbles these close-packed particles. Their positions are slowly randomized, their mutual close fit is lost, and the foundation steadily swells as its micro-structure opens up. Like a soft mushroom pushing up a paving stone, the shattered but confined foundation lifts the road surface above it, exactly counteracting the slowly settling earth beneath. The road will remain flat and stable and safe. Thanks to DREADCO's new shoddy concrete, Britain's shoddy roads will effortlessly shake off the European heavyweight challenge.

Vibro-expansive concrete will be welcomed not merely by road builders, but by civil engineers of every kind. Urban buildings shaken by nearby traffic, factories full of vibrating machinery, railway lines and airport runways, all will be cheaper and more stable on their new crumbling foundations. Tottering cathedrals, traffic-shaken monuments, and the like, will also benefit. Just inject the new concrete into the crucial gaps and cavities, and let the traffic do the rest. Daedalus has sent details of the process to the authorities at Pisa.

David Jones

DNA fingerprinting on trial

SIR—Two points arise from Lander's interesting Commentary "DNA fingerprinting on trial"¹. First, Lander describes Lifecodes's use of a criterion for comparing measurements on two profiles whereby bands that are within 3 standard deviations (s.d.) of each other are deemed to match. The weaknesses of this kind of approach in the context of forensic science were first exposed by Lindley² more than 10 years ago. An arbitrary cut-off, whereby a difference of 2.95 s.d.s results in positive evidence whereas one of 3.05 s.d.s leads to an exclusion, makes something of a lottery of a forensic comparison. Furthermore, to apply the criterion in isolation from the population data is artificial: whereas we may feel comfortable about reporting an exclusion at 3.05 s.d.s when the bands being compared are at a very common region of the population distribution, the decision looks dubious when they are in a sparse region. There is another problem with the application of this criterion to restriction fragment length polymorphism analysis: because errors in band positions within a profile are highly correlated, to apply two independent 3 s.d. tests to the two bands is incorrect.

Lindley's solution, albeit to a different type of evidence, is to calculate the Bayesian likelihood ratio. In this way, in the present context, the band measurements to be compared would be combined with the relevant population data in a function which will change smoothly from relatively large values, if bands are close, to progressively smaller values the further apart they are. Values of the likelihood ratio greater than one will support the explanation that the two profiles have the same origin, whereas values less than one will support the explanation that they have different origins. It is straightforward to handle correlated errors.

Second, Lander explains the process of using the frequencies of bands occurring in windows of arbitrarily selected size to estimate match probabilities. Deciding on the correct window width is problematical as we have discussed elsewhere³. We believe that it is preferable to estimate the underlying probability density function by kernel density estimation, in a manner similar to that used by Evett *et al.*⁴ in the analysis of another bivariate forensic problem.

Nature's Opinion⁵ on the issues raised by Lander said "The underlying difficulty is that science and the law differ in temperament." We agree entirely. Forensic scientists continually strive to bridge this temperamental divide which means that forensic science has a unique disciplinary status. Statistical practices which are successful in other scientific arenas are not

necessarily suitable within forensic science. Our approach to the analysis of data from a single locus, based on Lindley's principles², has recently been outlined at an FBI symposium⁶.

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SN1987A pulses

SIR—The reported 8-hour periodicity¹ in the emission from the pulsar in SN1987A may be due to the presence of a companion object^{1,2}, but the companion would somehow have to have been created after the explosion. Long period modulations due to pulsations in the neutron star itself can be excluded². Another possibility is that the 8-hour periodicity results from 'shaking' of magnetic field lines near the region of optical emission. For pulses at either the rotation frequency or the lowest radial vibrational mode³ and nominal models of the neutron-star structure^{4,5}, there is the possibility of exciting long-period hydromagnetic modes which involve global oscillations of the neutron star with periods of about 8 hours. But such oscillations cannot account for the observed amplitude of the oscillation shift without being highly nonlinear in the associated displacements and magnetic field perturbations¹. Most of the frequency variation, if it is real³, probably results from another effect.

There is a long-period 'hydromagnetic inertial' mode for which magnetic tension is balanced by the Coriolis force in the rotating frame^{5,15} (the Lense–Thirring effect modifies the quantitative details of this mode for realistic neutron-star models⁶). This mode occurs if the rotational speed at the star surface is small compared to the 'average' Alfvén speed through the star (oscillations of the superfluid vortex lattice yield even longer periods⁷). The associated shaking of the magnetic field lines in the emission region could affect the beaming of the pulses, and, hence, their intensity^{8,9}, which could explain observed variations in the luminosity¹.

For a constant density sphere with a constant, spin-aligned magnetic field, the oscillation frequency of the lowest (torsional) normal hydromagnetic inertial mode is^{10,11} $\omega \approx (8.9/2\Omega)(V_A^2/a^2)$ where a is the radius of the oscillating sphere, Ω the spin frequency and V_A the average Alfvén speed.

The superconducting core protons are coupled to the magnetic field via the normal electrons, and the proton mass density is ~ 0.05 of the neutron mass density¹²; if the more massive neutron core is coupled to the magnetic field via the protons and/or electrons on a timescale much less than 8 hours¹² then the Alfvén speed through the core will be smaller by a factor ~ 4.5 for the same magnetic field strength than if the coupling time were relatively long¹³.

With $\omega = 2\pi/(8 \text{ h})$, $\Omega \approx 1.2 \times 10^4 \text{ s}^{-1}$ and 'average' density $\sim 1.5 \times 10^{15} \text{ g cm}^{-3}$ out to $\sim 7 \text{ km}$ (from model G of ref. 6), we obtain a magnetic field $B \approx 7.4 \times 10^{13} \text{ G}$. This value decreases to $\sim 1.6 \times 10^{13} \text{ G}$ if only the protons in the core provide the effective mass density. But the observed luminosity limits the magnetic field to $\sim 10^9 \text{ G}$ (refs 1,3).

Such a small field may still be in agreement with a global oscillation. As the tilt angle of the magnetic field is increased with respect to the spin axis, components of the governing equation of motion tend to be decoupled for short wavelength oscillations¹⁵. We can estimate a fundamental mode (Alfvén) frequency as $\omega \approx \pi V_A/a$. The implied field is then in agreement with the luminosity limit: $\sim 6.7 \times 10^9 \text{ G}$ or $1.5 \times 10^9 \text{ G}$ (ref. 16) (no neutron coupling).

For the oscillation to remain linear, the maximum angular displacement of the crust should be limited to ~ 0.1 turn (~ 0.6 radians). Hence, for a torsional oscillation with a period of 8 hours, we require $\Delta\Omega \leq \sim 1.3 \times 10^{-4} \text{ s}^{-1}$, which is smaller than the observed variation by a factor of about 70. This limit is within a factor of four of residuals found in a best fit to the data but is also within the range of uncertainty in the frequency itself¹. For these modes to account for the observed frequency modulation, the magnetic field would be twisted through several complete revolutions. The frequency would then be much larger than implied by the linear treatment, but, it is not clear that the lattice structure of the magnetized vortices could be neglected in such an extreme case.

In the radial-vibration model³, rotational effects still dominate the propagation of hydromagnetic waves. The magnetic field values inferred above for a 0.1-second period are $5.3 \times 10^{12} \text{ G}$ and $1.2 \times 10^{12} \text{ G}$ (again, depending on coupling in the core), consistent with the observed luminosity.

If the radial pulsation frequency is

greater than the spin frequency, stratification can be important¹⁵. In the case $\omega_{\text{vib}} \gg \Omega \gg V_A/a$, the low frequency hydromagnetic waves have a more complicated character. Taking $\omega_{\text{vib}} \equiv |\mathbf{N} \times \mathbf{k}|/\kappa$ (N is the Brunt-Väisälä frequency¹⁵), frequencies of the short wavelength modes (wave vector $\mathbf{k}$) are $\omega_{\pm}^2 \approx \omega_{\text{vib}}^2 + (2\Omega \cdot \mathbf{k}/\kappa)^2 + (\mathbf{V}_A \cdot \mathbf{k})^2$ and $\omega_{\pm}^2 \approx (\mathbf{V}_A \cdot \mathbf{k})^2$ (For $N = 0$, ω_{-} becomes the hydromagnetic inertial mode). Purely Alfvénic oscillations are decoupled from the rotation frequency, and the inferred field strength is again $\sim 5 \times 10^9$ G.

Vibrational modes could, in principle, remove the phase accumulation problem via the beating of two closely spaced modes ($\Delta\omega = 2\pi/8$ hours). However, hydromagnetic effects and rotation lead to splitting at the rotation frequency¹⁴ and this only when the Alfvénic oscillation frequency dominates as in the surface layers of the star.

Note added in proof: Purely Alfvénic oscillations as a cause of the 8-hour periodicity have also been noted by Katz¹⁶.

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Sulphate aerosols and climate

SIR—One of Schwartz's¹ conclusions is that the cloud component of the albedo of the Southern Hemisphere (SH) is substantially greater than that due to clouds in the Northern Hemisphere (NH). He asserts that this difference, being of opposite sign to that which would result from the presence of anthropogenically elevated quantities of cloud-condensation nuclei (CCN) in the NH, contradicts the thesis of Charlson *et al.*² Schwartz's second argument depends on his assertion, taken from elsewhere³, that warming trends this century show "no discernible interhemispheric difference".

There are many factors known to affect temperatures. We have shown (for example, ref. 4) that Northern American cloud amounts have increased this century by as much as 10 per cent. Such an increase in cloud amount could, of course, be associated with increased concentrations of

CCN in the United States, Canada and the Arctic and thus compound the cooling for which Schwartz is searching. On the other hand, these clouds could be predominantly cirrus type and hence augment the greenhouse heating and perhaps thereby compensate for Schwartz's hemispherically asymmetrical CCN. We do not know how cloud amounts have varied this century, much less whether cloud types have altered. It seems foolhardy, at present, to assume that all other features of the climate system except CCN and greenhouse gases, have remained hemispherically symmetrical and unchanging this century. Without this assumption, which is highly questionable, and without temporal analysis of CCN variations, Schwartz cannot, in our opinion, deduce anything from hemispheric temperature trends.

Schwartz uses Ellis⁵ as the source for latitudinal cloud amounts, F_c , and Berlyand

*et al.*⁶ for the total planetary albedos, α_T , and the clear-sky planetary albedos, α_c . Ellis's data are an inadequate source of statistics on Earth's albedo. The values used by Schwartz are derived from eight weeks of Nimbus 3 satellite data recorded during four two-week periods in 1969. For one of these periods, most of the Pacific Ocean north of 45° was not sampled. There are now more complete and more accurate data sets of satellite-measured albedos.

Similarly, the data of Beryland *et al.*⁶ are a sound, valid estimate of global cloud cover. At the time of its production it was arguably the best global cloud climatology but it has naturally been superseded by very many satellite-based nephanalyses and global archives of surface-based observations of clouds (for example, ref. 7).

We think that Schwartz should have compared the curves of planetary albedo, α_T , in his Fig. 1. These curves show that NH = SH at the equator, NH > SH at 5°, SH > NH at 15°, NH > SH at 25°, equality at 35° and 45° and SH > NH in the remaining four latitude bands, 55° to 85°. The differences are less than 0.02 and seem to alternate between 0° and 60° (poleward of 60° both the cloud and albedo data are highly suspect). Thus, in some latitudes the hypothesis of Charlson *et al.*² seems to be validated by Schwartz's Fig. 1.

Schwartz does not, however, examine the total planetary albedo differences; rather he considers the "cloud component of the albedo". The curves plotted by Schwartz are of the quantity $\alpha_c F_c$, where F_c is the fractional cloud cover, derived directly by subtracting the product $(1 - F_c) \alpha_c$. This term is itself a product of cloud amount and cloudy sky albedo. Schwartz derives larger values for this product in the SH for most latitudes which indicates either that F_c is greater in the SH, that α_c is greater in the SH, or both. The cloud amounts, F_c , which Schwartz uses are indeed generally larger in the SH and

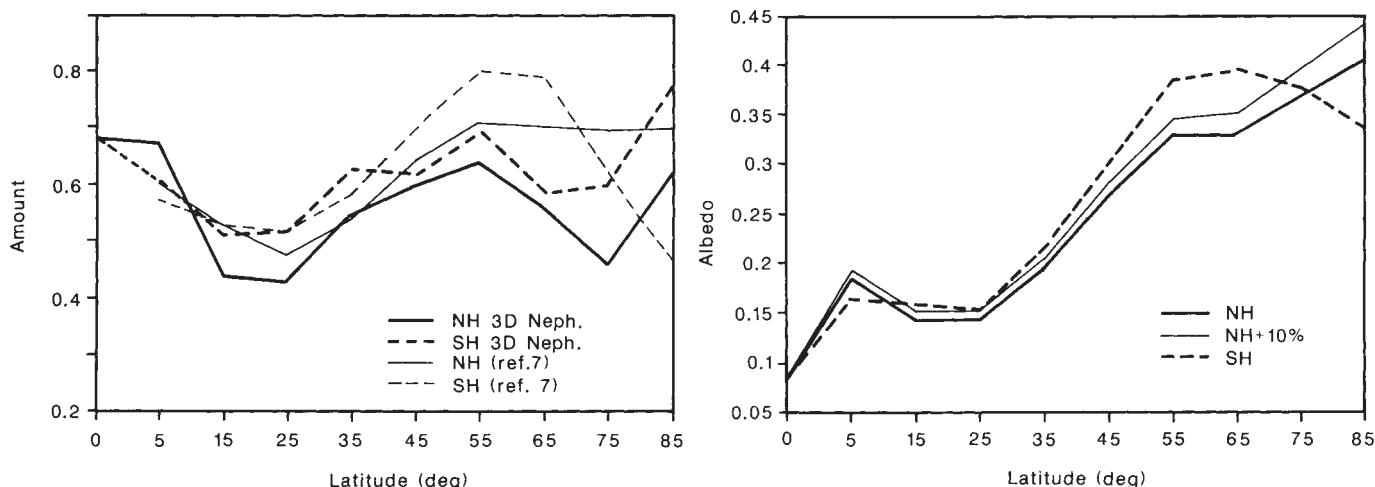


FIG. Latitudinally averaged values of total cloud amount derived from Berlyand and Strokina⁶ and the three-dimensional nephanalysis⁸ derived predominantly from surface-based observations for many years and satellite-based observations for 1979, respectively. Note that the satellite-based cloud amounts are latitudinally more coherent and that both climatologies have SH cloud > NH cloud in most latitudes.

other global nephelyses⁸ also tend to have greater cloud cover in the SH (see figure). That the latitudinal cloud amounts differ rather considerably from one another in the figure is not surprising when it is recognized that the USAF three-dimensional nephelyses is predominantly a satellite archive for 1979 whereas the Berlyand *et al.* data are predominantly surface-based and accumulated over a much longer period. In any case, it is well known that evaluations of latitudinally averaged values of total cloud amount differ by at least $\pm 10\%$ (absolute cloud amount)⁹. Thus if Schwartz had chosen to use a different source for his F_c values, the $\alpha_c F_c$ curves in his Fig. 1 would have been modified. Moreover, these curves are highly suspect as they represent the planetary albedo as viewed from the top of the atmosphere in areas designated, from another incompatible data source, to be cloudy.

We conclude that the hypothesis of Charlson *et al.* has not been refuted.

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STR—Claiming that man-made emissions of SO_2 have had no effect on surface-temperature trends or planetary albedo, Schwartz¹ attempts to refute our hypothesis² of a biological mechanism for climate regulation. His arguments are flawed for several reasons.

First, although Schwartz interprets the analyses of Jones *et al.*^{3–5} to show that the changes in surface temperature in the past century for the Southern Hemisphere (SH) is not significantly greater than that for the Northern Hemisphere (NH), Jones *et al.* have themselves also said⁶, “the warmth of the 1980s is most evident in the Southern Hemisphere”. That statement refers to a single decade only, but an independent analysis⁷ has extended it back through two more decades and concluded that the 20-year trend of surface air temperature (1958–77) over the oceans has a latitudinal dependence, with the SH warming more rapidly.

We do not know why different analyses of the historical record of surface temperatures lead to opposite conclusions about the trends. But even if those studies were to be reconciled they might still not offer a clear test of the effect of sulphate aerosol on cloud albedo, because there are probably climate factors other than cloud albedo that could cause the temperature trends to differ in the two hemispheres. (See also Wigley⁸.)

Using a published cloud climatology and published measurements of planetary albedo, Schwartz also concludes that average cloud albedo is no greater in the NH than in the SH. He takes this as evidence against an influence of industrial sulphur pollution on cloud albedo, despite the fact that the lifetime of sulphur in the atmosphere is typically not more than a few days, so the effect of pollution-derived aerosol may not extend much beyond the NH continents. Therefore it is likely that the geographical footprint of the pollution aerosol does not greatly overlap that of the sensitive marine stratus clouds, particularly in the subtropics.

However, sulphate aerosols, whether derived from industrial pollution or from dimethylsulphide, can be expected to influence only one of the determinants of cloud albedo, namely the effective cloud-droplet radius, r_{eff} . As Stephens⁹, for example, has shown, cloud albedo is a function principally of three variables: vertically integrated liquid water content (liquid water path, LWP), solar zenith angle, and r_{eff} . It is unlikely that the average LWP of clouds in the SH is the same as in the NH. As the average LWP for each hemisphere is unknown, the effect of sulphur on r_{eff} cannot be inferred just by comparing hemispheric-average albedos, as Slingo¹⁰ emphasized. Long-term trends in planetary albedo are also not available, even though they have been measured for three decades, because of changes in the satellite instruments.

Much of Schwartz's argument is based on a comparison of non-sea-salt sulphate (n.s.s. SO_4^{2-}) concentration over the oceans in the Northern and Southern hemispheres. He compares measurements made by a variety of authors with a variety of techniques. Unfortunately, there is considerable uncertainty about the accuracy of such measurements. Very few sampler intercomparisons have been made, none of them conforming to the rigorous standards that are now expected to be part of intercomparison protocols. Often the large size fraction ($>1\text{--}2\text{ }\mu\text{m}$, depending on the size cut of the sampler), which typically contains about 30–40 per cent of n.s.s. SO_4^{2-} , is either not collected or its n.s.s. SO_4^{2-} content is ignored. Samples of fine n.s.s. SO_4^{2-} and total n.s.s. SO_4^{2-} should therefore not be compared, as has been done by Schwartz¹.

If we take the data in Schwartz's Table 1

at face value, we see that it contains data from both ‘dusty’ and ‘clean’ episodes in the North Pacific and North Atlantic. Much of the sulphate in the dust episodes is actually on coarse particles and therefore provides few cloud condensation nuclei (CCN). During the ‘low-dust’ season, Barbados shows about the same values as Samoa, and the summer Arctic and North Sea trajectory data from Velen are about the same as Cape Grim and Punta Arenas. We conclude that many of the NH sites are about as clean as the climatically and biogeographically comparable SH sites for much of the time. The only significant difference arises during dust transport events (where much of the SO_4^{2-} is coarse and does not provide many CCN) or at some obviously and expectedly polluted sites — for example, Bermuda. Much of the dust transport is of course natural, and would not contribute to a potential time trend. It seems that anthropogenic inputs are significant for boundary-layer n.s.s. SO_4^{2-} levels only over the Atlantic north of 30°N , and possibly over the northern North Pacific, but sulphate distributions there are presently not well known. Looking at our own cruise data from the tropical Pacific, we find that the NH n.s.s. SO_4^{2-} data¹¹ are actually lower than the SH and equatorial ones. Consequently, 40 per cent at most of the area over the NH oceans might experience significantly elevated CCN concentrations.

The aircraft data from the North Pacific off Washington State and the south Indian Ocean off Tasmania do suggest significant differences in n.s.s. SO_4^{2-} levels^{12,13}. But the total aerosol sulphur in the atmosphere is comprised of sulphate and methanesulphonic acid. Thus, when methanesulphonic acid is added to n.s.s. SO_4^{2-} , the total aerosol sulphur in the sub-cloud mixed layer is essentially the same for the Tasmania and Washington data sets. We know that the size distributions of methanesulphonic acid and n.s.s. SO_4^{2-} are similar, and we may assume that they are internally mixed, and that both contribute to the mass of soluble particles, and thus to CCN active at low supersaturation. But in the free troposphere there is a pronounced difference between the two ▶

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data sets, with levels over the North Pacific substantially elevated relative to the south Indian Ocean. It is not clear, however, just how far two experiments of only about two-weeks duration each can be extrapolated, and how much the aerosol at these altitudes contributes to cloud formation.

Finally, we note that Schwartz² did not attempt to test the hypothesis that most natural marine CCN are produced from dimethylsulphide. Regardless of any climate role of CCN, understanding their sources, properties, distribution and variability are important objectives.

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SIR—Schwartz¹ proposes that because aerosol sulphate concentrations are considerably higher in the industrialized Northern Hemisphere (NH) than in the Southern Hemisphere (SH), the climatic effect should be observed in the difference between the hemispheric temperature trends. Based on data presented by Jones *et al.*^{2,4}, he reports that there is no difference in the temperature trends of the two hemispheres over about the past century and therefore no supporting evidence for the role of SO₂ in climate.

We do not think that such a conclusion is justified given the sparsity of temperature data, particularly for the SH, earlier this century. A recent analysis of the spatial patterns in temperature trends for 1947–86 in the NH and in the SH (data for the Antarctic were not available until the late 1950s) points to almost uniform warming in the SH and large regions of both cooling and warming trends in the NH⁵. Earlier studies also indicate regions of positive and negative temperature anomalies in the NH^{6–8}. Given the large geographic variability of sulphate concentrations, one could expect its impact to be regional. Among the areas which exhibit cooling are eastern North America and Europe, both known to be regions of exceptionally high SO₂ emissions that have increased substantially over the past century. Large regions of cooling are also observed over the North Atlantic and North Pacific oceans⁵. Qualitatively, the

observations are not inconsistent with the idea of regional compensation of CO₂ warming by aerosols. Therefore, given the complexity of the climate system, the question of the influence of sulphates on climate remains open.

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SIR—For a link to exist between changes in gaseous sulphur emissions and changes in planetary albedo several conditions must be met. The conditions associated with air masses containing anthropogenic SO₂ emissions may not be conducive to changing cloud albedos. First, increases in concentrations of cloud condensation nuclei (CCN) must necessarily lead to increases in typical cloud-drop concentrations and decreases in drop size. Such an assumption is consistent with observation when CCN concentrations are low — as expected over cleaner areas in both the Northern hemisphere (NH) and the Southern Hemisphere (SH). But in areas where the CCN concentration exceeds 1,000 cm⁻³ and where sulphate concentrations exceed 2,000 ng cm⁻³, this relationship is not observed². Such a lack of correlation is expected because as CCN concentrations increase, supersaturation levels in clouds become depressed, causing a smaller fraction of aerosol particles to act as CCN³. Because continental air masses typically contain plenty of CCN, addition of anthropogenic sulphur is not expected to affect the cloud-drop number density in these air masses.

Second, the measurements of sulphate concentration given by Schwartz¹ are in terms of total mass per unit volume; no measurements for number density of sulphate particles are provided. Schwartz assumes that sulphate concentrations would be proportional to CCN concentrations and that the increased sulphate concentrations observed in the NH would imply increased numbers of CCN. This would only be expected if the conversion of anthropogenic SO₂ to sulphate were dominated by gas-phase processes followed by homogenous nucleation of SO₄²⁻ to the

aerosol. In fact, a significant proportion of SO₂ is converted to sulphate via aqueous reactions which take place in cloud drops. These reactions would increase the size of existing aerosol and CCN particles but not their number. An increase in size is observed in air masses which originate over continents and move offshore⁴.

Satellite observations presented by Schwartz¹ indicate that the cloud component of the planetary albedo is larger in the SH than in the NH, which he says is opposite to the difference expected if sulphate controls the number of cloud droplets. However, he implicitly assumes that the cloud fraction is the same for each hemisphere, which is not the case. The fractional cloudiness of the SH is generally larger than that of the NH⁵, so that the cloud albedos are not necessarily greater in the SH. An additional shortcoming is the lack of distinction between marine and continental clouds, whose albedos are influenced by the difference between the albedos of the underlying surface. Moreover, the liquid water content of clouds varies widely with latitude and altitude. If satellite measurements of planetary albedo are to be used to evaluate the influence of sulphate aerosols on cloud albedo, comparisons between marine clouds of comparable liquid-water content must be performed.

Finally, the observed albedos of optically thick clouds are significantly lower than expected from a straightforward theoretical analysis. One explanation for the discrepancy is the absorption by graphitic carbon particles that have been scavenged by the cloud drops⁶. This requires the carbon to be at the surface of the drop in order to substantially increase its absorption, but this has been observed in laboratory experiments⁷. The carbon, of course, is associated with urban pollution and would originate in the same continental regions as the anthropogenic sulphur discussed by Schwartz¹. For clouds of intermediate optical depth the effect of carbon on cloud albedo is much less significant, but the lowered albedo of optically thick clouds would still tend to obscure the analysis by Schwartz because he does not distinguish between optically thick and thinner clouds.

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Was Burt stitched up?

Steve Blinkhorn

The Burt Affair. By Robert B. Joynson. *Routledge: 1989. Pp.347. £25.*

IT is hard to imagine a project less likely to attract universal enthusiasm in the world of academic psychology than an exculpatory account of the Burt affair. After the scandal of the initial claims in the *Sunday Times* that the late Grand Old Man of British psychology had manufactured data and research assistants wholesale came a damning biography by Leslie Hearnshaw which appeared to set the seal once and for all on a most shameful reputation. As salutary a tale of scientific fraud has scarcely been told.

The conclusion was so damning, the evidence apparently so conclusive, the fraud so transparent, the circumstantial suggestions of similar but less well-documented transgressions so frequent and from such well-connected sources that perhaps no-one thought to retrace all the ground or to examine all the accusations in detail. Not, that is, until Robert Joynson accidentally came across an inconsistency in the case for the prosecution that had never really been followed through.

The tale he tells in his account of the Burt affair is no less salutary than the original scandal. It has radically shifted my view of the matter, and of my willingness to accept the judgement of my elders and betters. Joynson has not, it should be said straight

away, discovered conclusive evidence that Burt never strayed a millimetre from the most rigorous modern standards of scientific research and reporting. He has not found the mysterious Misses Howard and Conway living comfortably in retirement homes. But he has produced a story about the supposed fraudulent concoction of data concerning IQ and kinship which makes sense to a reader who never knew the principals in the drama. What is more, he has demonstrated that the standard of reporting in Hearnshaw's biography is seriously deficient in areas tangential to the main argument, but areas drawn upon in formulating what is currently the official story.

It seems to me that verdict was pronounced, and then more evidence sought or reinterpreted in support of that verdict. If Joynson is correct, then an injustice has been done, and Burt's accusers are guilty of selective reporting and misreporting.

It is a dogged book, attending to fine detail in a way the subject demands, but the broad outline can be sketched fairly

IMAGE
UNAVAILABLE
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REASONS

Cyril Burt: claims that he was guilty of manufacturing "data and research assistants wholesale" are now re-examined, raising the possibility that he was the victim of injustice and "selective reporting and misreporting" by his accusers.

quickly. Joynson's tentative outline of the history of the kinship correlations is roughly as follows. Very nearly all the data Burt drew upon were collected before the Second World War, although perhaps a few cases of separated monozygotic twins were brought to his attention later. He used his continuing contacts at London County Council for the fieldwork, perhaps including Howard and Conway. On the evacuation of his department to Wales, he extracted these data, but in the confusion some considerable part was misfiled.

After the war, and his retirement, he set about working his data into publishable papers, only to find that many of the twin

data were missing. When they were rediscovered, he felt unable to ask his secretary to type a paper for publication explaining that she had been so incompetent as to mislay such vital data for ten years or more. He also felt it only proper to credit his pre-war (and probably unpaid) assistants for their work, despite the fact that he had lost touch with them. In many cases — such as the physical variables he had investigated as well as IQ — he simply included previously calculated correlations despite the fact that no further data had come to hand, so these are identical across the various publications. He failed adequately to indicate the sample sizes for each published coefficient. On his death,

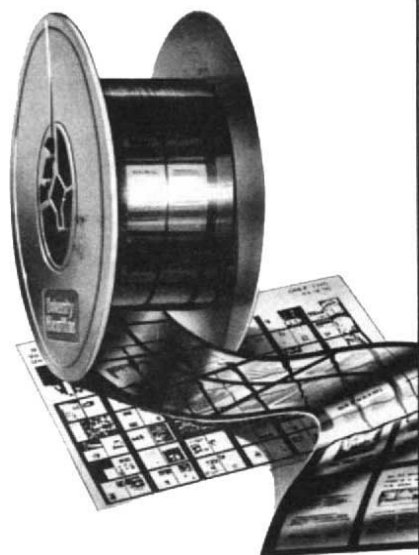
files possibly containing the relevant data were not extracted by his biographer; indeed, on the advice of Liam Hudson, they were actually destroyed.

I do not know whether I believe this story, but I find it as believable as the notion that a major figure in statistical psychology would sit inventing data to make correlations stay constant despite a change in sample sizes (which is one of the main thrusts of the accusations against him). Just who did he think would be fooled? I do know from long experience that the behaviour of correlations based on psychological test data can be very counterintuitive, and that doing correlational analysis by hand is tedious and error-prone, a fact easily forgotten in these days of readily available computer power.

No review could do justice to the detail in which Joynson discusses these matters, or to the complexity of the arguments either way. But there is at least an argument for the charitable

view that Burt's malpractices were many and minor, and that they only assume the aspect of grand fraud if you have reason to want to discredit him. It is harsh to expect a pioneer to subject his life's grand enterprise to critical standards that only gained wide acceptance after most of the fieldwork was done, and perhaps unreasonable to expect an old man to be able to. Which is not to say that his results, even if genuine, contribute any longer to our knowledge. His methods were, by modern standards, too subjective, too badly documented, insufficiently rigorous and inadequately reported. But subsequent, far better-conducted research, has yielded broadly similar results, and the Burt

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scandal has not disposed of the nature–nurture debate, fruitless controversy though it be.

More interesting than the ‘did he, didn’t he?’ aspect of the story is what the world made of Burt once guilt had been presumed. There have been many subsidiary accusations of malpractice from those who knew him, but from the small number I have had leisure to look into independently, I suspect Joynson is right in concluding that their significance has been exaggerated in the aftermath of the scandal. The ‘articles’ which Burt is supposed to have published under the names of A. D. B. Clarke and Ann Gravely (later Clarke), for instance, turn out to be clearly labelled as thesis abstracts, and although I have not read the theses themselves, as Joynson has, I can find nothing in the abstracts to suggest heavy distortion.

More important from a historical point of view is the question of Burt’s contribution to the development of the technique of factor analysis and his claims regarding his own role, which, according to Hearnshaw, were “contrary to the evidence”. It so happens that I read much of the early published work in this field when working on my own PhD thesis, and had always had the impression that Burt had not received the recognition he deserved.

Now it is the case that most often Spearman is credited in textbooks with first introducing factor analysis, and Thurstone with what is called multiple factor analysis, the technique much in fashion at one time for the investigation of the structure of mental ability. But not everyone who writes a textbook relating these credits is an expert in the field, and of those who are, most are American.

To non-specialists, the details of Spearman’s tetrad equation, the difficulties of applying it and its eventual obsolescence must be obscure. Certainly, Spearman introduced the notion of factors as explanatory variables into psychology. But when it comes to practical techniques, Burt was the real innovator. His “simple summation” method was practical, effective and at the very least anticipated Thurstone’s methods. The point about Americans is to do with the belief still current in some quarters in the United States that there is a London school of what are sometimes called British factorists, of whom Spearman was the leading light, insisting on the existence of a general factor of intelligence, and methodologically at one. The appearance of unity is often enhanced by distance. What divided Spearman and Burt on the one hand from Thurstone on the other was belief in the reality of *g*. But Thurstone’s methods were far closer to Burt’s and share a common mathematical basis.

Burt eventually credited Pearson with

the basic ideas, citing a paper published in 1901 as containing the essentials, although it was a data paper by Macdonell in a different journal in the same year that seems to have been the spur to his interest. Hearnshaw seems to have assumed that a reference by page number to the Macdonell paper was in fact a reference to a paper by Pearson in the same journal.

Joynson has not read the crucial paper by Pearson because he cannot follow the mathematics, so he cannot say whether it is consistent with Burt’s account. I have; I can; it is. It develops the idea of representing correlational data by the principal axes of an ellipsoid. This is the crucial notion of multiple factor analysis. True, Pearson does not add “and what a jolly idea it would be to apply this method to mental tests”, but anyone familiar with how factor analysis actually developed into a widely used method would recognize this paper as the *fons et origo*. Indeed, it seems to me that Burt in retrospect would have every right to feel rather peeved that his own contribution had gone unrecognized. Guilford, in his 1936 book *Psychometric Methods*, makes no mention of Burt, only “Spearman and his students”, when discussing distinctive elements that Burt introduced into factor theory. Burt was never Spearman’s student. Later texts have often taken Guilford’s authority as sufficient for their own accounts.

Now none of this really matters from the point of view of modern practice, but academics do get upset about priority and attribution and have been known to take great pains to ensure they get credit for work they have done. Hearnshaw would appear to have been less than diligent in reference to primary sources, and insofar as I have been able to follow up Joynson’s line of enquiry I find it meticulously accurate. It was doubts about Hearnshaw’s dependability on this historical issue that led Joynson to widen his enquiry, and to a painstaking re-examination of the rest of the accusations about Burt. Even so, it is difficult to tell how comprehensive and objective his account is, despite the great detail of his investigation.

I was left wondering how much minor fudging (intentional or otherwise) goes undetected, and who among us will leave such a well-documented archive that *post mortem* accusations of fraud could not be brought. All the world loves to hate a villain, the more so if his villainy is seen to be total. I do not suppose that this book will settle the Burt controversy, but at least it raises some pertinent questions which deserve answering at the same level of detail. ☐

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Being on the shelf

Graham Harris

Ecosystems of the World 27: Continental Shelves. Edited by H. Postma and J. J. Zijlstra. Elsevier:1988. Pp.421. Dfl.360, \$189.50.

CONTINENTAL shelves account for only about seven per cent of the surface area of the world's oceans. That figure belies their importance, however, which is due not only to their proximity to human influences but because about 90 per cent of the world's fishery resources are harvested on shelves. So an understanding of the basis of their productivity and interannual variability is of great practical and economic significance.

The book reviewed here begins with seven chapters giving an overview of the physical, chemical, biological and geological characteristics of shelf systems; the last four present detailed case studies of Arctic, tropical and temperate systems. Generally, the perspective is a global one, but there is a decided bias towards examples taken from northern temperate waters, especially the North Sea. Further, although the editors perhaps felt that any explicit discussion of human influences should be restricted to commercial fishing, the book would have been considerably improved by inclusion of a contribution on coastal eutrophication, 'red tides' and other pollution effects from river discharges and the disposal of wastes at sea.

The most useful chapters are those which provide summaries of the relevant physics and geology, together with those on the flows of energy in pelagic systems and the fluctuations in fish populations and commercial fisheries. The examples given show that shelf systems exhibit much interannual variability, and we get a clear view of the general inability of ecological theory to account either for the overall fisheries yield of shelf waters or for the interannual variability in those yields. Cushing provides a particularly good review of the types of production in shelf systems and the ecological efficiencies observed at various levels in the food chain.

What is not clear, even in well-documented systems such as the North Sea, is the extent to which long-term changes in fisheries yields and catch compositions are due to changes in primary production, changes in energy efficiencies at various levels in the food chain, structural changes related to the physical environment or changes in biological interactions due to fishing activity. Equilibrium 'fixed engine' models will certainly not do. Sharp's

excellent chapter on fluctuations in fish populations and fisheries rejects a generalist approach in favour of a discussion of the ecological relations between fish, habitat and climates. He reviews the global ecology of fish populations in continental shelves, and ends by stressing the unpredictability of population interactions in fluctuating environments and calling for more realism on the part of fisheries managers.

The first of the four case studies is a comprehensive review of the shelf ecosystem of the north-east United States. Again, the long-term trends in fish biomass and species composition cannot be clearly attributed to the effects of fishing, to predator-prey interactions or to environmental factors — multiple correlation analysis of 22 species of fin-fish and squid revealed little more than might be expected by chance.

Overall, the reader gets a strong

impression of the large volume of research that has been carried out on shelf systems all over the world. Much descriptive information has been gathered about biogeography, the composition of pelagic and benthic communities, and the physical, chemical and geological basis of the ecosystems. What is singularly lacking is a theoretical basis for generalizations and predictions about the ecological processes and interactions which produce the observed interannual variability. For many purposes, the management of shelf systems requires predictions of the effects of human activities in the face of variability in climate. Although this book was long delayed in production, meaning that there are few references dated after 1984, it clearly reveals the shortcomings of present approaches. □

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A cooler view

V. J. Emery

Copper Oxide Superconductors. By Charles P. Poole Jr, Timir Datta and Horacio A. Farach. Wiley: 1988. Pp.289. \$35, £27.85.

A SUDDEN and dramatic development in any field of activity will surely spawn its own literature, the more so when it captures the public imagination and lures thousands of researchers all around the world. High-temperature superconductivity is no exception.

First to get their books to press were the popularizers who chronicled the 'Woodstock' atmosphere of the 1987 March meeting of the American Physical Society or the race to unravel the structure of the first liquid-nitrogen-temperature superconductor. Now, with the passage of time, books designed for a more involved scientific readership are starting to appear. One of the first is *Copper Oxide Superconductors* which surveys the results of some 12 months of round-the-clock research, mainly in 1987. The scope is avowedly experimental, although there is some discussion of theories, ideas and explanations "to give coherence". The outcome of this attempt at an instant review of more than one thousand papers is not surprising: although there is much of value, the presentation lacks the depth and perspective of a more considered work. It is a sourcebook rather than a textbook.

The authors come closest to attaining their goal in the sections on preparation, characterization and general structural properties. Many people will find it useful to have quick access to the basic informa-

tion and a convenient listing of the original work. Although it could not include a number of recent developments, particularly in the preparation of thin films and single crystals, the book does encompass the early body of knowledge.

But it is much more difficult to give a satisfactory account of the measurement and interpretation of physical properties. These are the stuff of controversy. Early experiments were carried out before sample preparation and characterization were fully under control, and it has taken some time to develop an agreed body of data. Early theories were not worked out in enough detail to allow an unambiguous and universally accepted explanation of what is going on. The evenhanded approach adopted by the authors gives an accurate impression of the very wide range of opinion that has been expressed, but does not give a perspective on the issues or a feeling for what must be done to achieve a consensus.

The book bears the mark of correction without revision: one is often arrested by misleading statements, only to find that some reparation is made towards the end of the chapter. But readers who are prepared to form their own opinions will find here an extensive compilation of data and a charted route to the original literature that will be invaluable. □

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● *Journey into Light: the Life and Science of C.V. Raman* by G. Venkatarman has now been published in Britain by Oxford University Press (£22.50). For review see *Nature* 338, 685; (1989). In addition, Oxford University Press has published *Scientific Papers of C.V. Raman*, containing his complete works in six separate volumes.

The simplest solution

Mark Ridley

Reconstructing the Past: Parsimony, Evolution, and Inference. By Elliott Sober. MIT Press: 1989. Pp.265. \$27.50, £25.75.

How do we know that chimpanzees share a more recent common ancestor with human beings than with amoebas? A less well-known phylogenetic group, such as chimps, humans and gorillas, or wasps, beetles and butterflies, poses the same question in a sharper form. The naive reply, that the answer must come from the fossil record, is of little help. We can already see that a chimp looks more like a human than an amoeba, and for any possible fossil we are again faced with the same question: how do we know that it shares a more recent common ancestor with chimps, or humans or amoebas?

In a general sense, the question is answered by the principle of parsimony. The best estimate of the phylogeny is the one requiring the smallest number of evolutionary changes. Parsimony has more and less exact meanings, and Sober's book is written at a professional level where these different meanings matter; but it is fair to say that all phylogenetic inference depends on some form of parsimony argument, or assumption.

And yet, it is far from clear that evolution is parsimonious. As Joe Felsenstein once remarked, "probably the most parsimonious outcome of evolution would be for it not to occur at all". In morphological evolution, examples of convergence are common, and in some cases the evolution of molecules can — even on the most parsimonious reconstruction — be very unparsimonious indeed. The question is not easy.

The various proposed justifications are the subject of Sober's book. Sober is a philosopher who studies biology, and he brings a rare skill to bear upon the philosophical arguments with which biologists (and others) have tried to justify parsimony. He describes the book as an interdisciplinary work, in philosophy, evolution and statistics, but I prefer to divide it into two main parts. Chapters 2 and 3 are philosophical and are concerned with simplicity and induction. Chapters 4–6 are more biological; they are about evolutionary models and aim to find out under what conditions the principle of parsimony provides a valid inference of phylogeny.

One reaction to the difficulty of justifying parsimony by evolutionary theory has been to deny that its justification is evolutionary at all. It is to be justified instead on philosophical grounds, by



Long in the tooth — scanning electron micrograph of rat-incisor enamel, showing two sets of prisms or rods at a 64–70° angle to each other and a third array of crystals lying perpendicular to the main rods. Scale, 2cm equals 10µm. The picture is taken from *On Biomineralization*, newly published by Oxford University Press.

Courtesy of Dr A. Jodalikin.

Ockham's Razor or some methodological preference for simplicity. A strange popperian justification has even been put forward, according to which the phylogeny requiring the fewest evolutionary events is best because it is 'least refuted'. Arguments of this kind have particularly appealed to one school of taxonomists, the 'pattern cladists' (who so vexed *Nature's* editorial writer a few years ago). Pattern cladists like to classify biological species according to their phylogenetic relations, but deny that this practice has anything to do with evolution. The reconstruction of 'phylogeny' (or whatever its non-evolutionary version is called) uses the parsimony principle, and pattern cladists are keen to find a non-evolutionary basis for it.

Sober will have none of this. He accepts that ("perhaps") it is now "the majority view" that scientists prefer simple hypotheses only for reasons of method, but he thinks this is a mistake. A preference for simplicity cannot be justified by methodology, and "an appeal to simplicity is a surrogate for stating an empirical background theory". I would go further. In the absence of evolutionary theory, I deny that a more parsimonious "phylogeny" is any simpler than a less parsimonious one. The parsimonious phylogeny does minimize the number of convergent characters and simplify the hypotheses about convergence. But it does so at the expense of ancestral characters, whose hypothetical numbers it maximizes. If you are trying to reconstruct phylogeny, there is a reason (parsimony) to treat shared characters as ancestral whenever possible; but in the absence of that aim an arrangement that minimizes 'convergence' is no simpler than one that maximizes it. (Convergence and ancestry are meaningless without evolutionary theory; we are just left with a list of characters and character states.)

So a scientific justification for parsimony is needed. Sober considers in detail two opposed arguments, those of Steve Farris and Joe Felsenstein, about the conditions

under which the parsimony principle gives the maximum likelihood phylogeny. Felsenstein showed that parsimony works on the assumption that evolutionary change is improbable. I believe this is a good reason for using parsimony, but Sober (and Felsenstein, it seems) is unhappy with it. Sober is mainly interested in establishing that Felsenstein's condition, on the arguments so far presented, is sufficient rather than necessary, and therefore not an assumption of parsimonious inference.

Farris's argument sounds less plausible to begin with. Suppose we have three species (A, B and C) and ten characters suggest the (A,B)C grouping and only one the A(B,C) grouping. Farris remarks that the evidence favours (A,B)C no matter how rare or common evolutionary change is. Even if evolutionary change is common, it is still more likely that at least *one* of the ten characters is shared from a common ancestor, and not convergent. As the probability of change increases, the one character linking B and C is devalued just as much as each of the ten linking A and B. This is true, but the question of reliability remains. The parsimonious inference may still be the best, but it has become less interesting. Felsenstein's condition has more biological appeal: if evolution is improbable, parsimony is reliable.

Many will find these issues excessively esoteric, and *Reconstructing the Past* is indeed written for fairly expert readers. Biologists (and philosophers, I imagine) with some previous knowledge will get something out of it. But the book is not conclusive; it offers a contribution, rather than a solution, to a research problem. It will, as such, be of most interest to the increasing number of people who are actually working on the statistical inference of phylogeny. Their philosophical appreciation of what they are doing should benefit from Sober's argument. □

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Association of class I major histocompatibility heavy and light chains induced by viral peptides

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We describe a cell in which association of a major histocompatibility complex class I heavy chain with β_2 -microglobulin is induced by a peptide derived from influenza nucleoprotein. Association of antigenic peptides with the binding site of class I molecules may be required for correct folding of the heavy chain, association with β_2 -microglobulin and transport of the antigen-MHC complex to the cell surface.

THE majority of cytotoxic T lymphocytes (CTL) recognize epitopes of viral or other foreign proteins in association with class I major histocompatibility complex (MHC) molecules¹. Epitopes are generated from protein antigens synthesized in the cytoplasm, and are presented at the surface of the cell in a form that can be mimicked *in vitro* by incubation with short synthetic peptides²⁻¹².

These results have led to the suggestion that the proteins recognized by class I-restricted CTL are degraded in the cytoplasm, and the peptides derived from them transported to the cell surface in association with class I molecules of the MHC^{2,3}. Although evidence has accumulated that is consistent with this hypothesis^{6,7,10,13-15}, the mechanisms by which epitopes are generated, transported and complexed with class I molecules are not known.

One approach to this problem is to study cell mutants which seem to lack one or more of these functions. We have investigated a tumour cell line that escapes immunological rejection *in vivo* across a minor histocompatibility barrier (Öhlén *et al.*, unpublished observations). It has a known defect in the association of β_2 -microglobulin with class I heavy chains, and a reduction of ~95% in the level of assembled class I molecules expressed at the cell surface¹⁶⁻¹⁸. This cell has lost the ability to present an epitope from influenza nucleoprotein synthesized in the cytoplasm, but does present the same epitope when exposed to it as a synthetic peptide in the extracellular fluid. This paradox is resolved by showing that exposure of the cell to extracellular peptide induces assembly, transport and surface expression of class I molecules. Association of peptides with the binding site on the class I heavy chain may be required for stable association of the heavy chain with β_2 -microglobulin, and expression of the peptide-MHC complex at the cell surface.

Endogenous antigen

The mutant cell line RMA-S was derived from the Rauscher virus-induced H-2^b lymphoma RBL-5 by exposure to the mutagen ethyl methane sulphonate (EMS) and repeated rounds of treatment with antisera against class I molecules and complement^{16,17}. It expresses ~1/20 of the amount of H-2D^b, K^b and β_2 -microglobulin at the cell surface when compared with RBL-5 cells exposed to EMS but not selected with antibodies (referred to as RMA). RMA-S synthesizes both class I heavy chains and β_2 -microglobulin, but most of the heavy chains bear high man-

nose oligosaccharides, do not associate with β_2 -microglobulin and remain intracellular¹⁸.

The mutant RMA-S was compared with RMA as a target for recognition by a cytotoxic T-cell clone (F5) specific for influenza nucleoprotein (NP) in association with the class I molecule H-2D^b (ref. 3). RMA was efficiently recognized and killed by clone F5 after infection with influenza virus, whereas RMA-S was resistant to lysis in identical conditions (Fig. 1a). The inability of this CTL clone to recognize infected RMA-S cells was not due to inefficient infection by the virus, as synthesis and degradation of NP in infected RMA-S were not impaired (data not shown).

RMA-S and RMA were then compared as targets after treatment with the peptide epitope recognized by the CTL clone

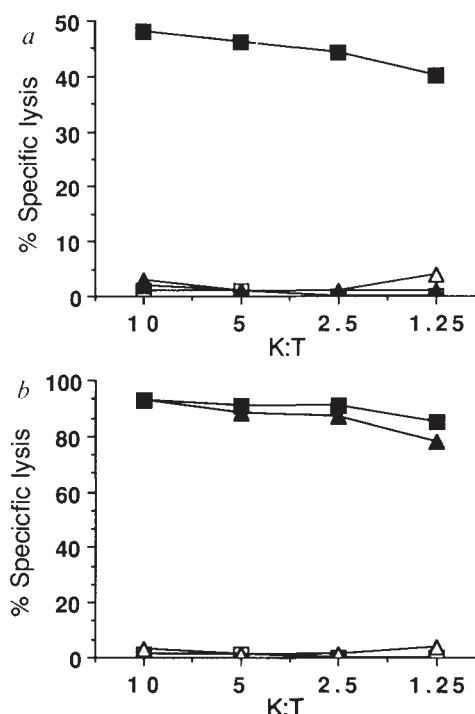
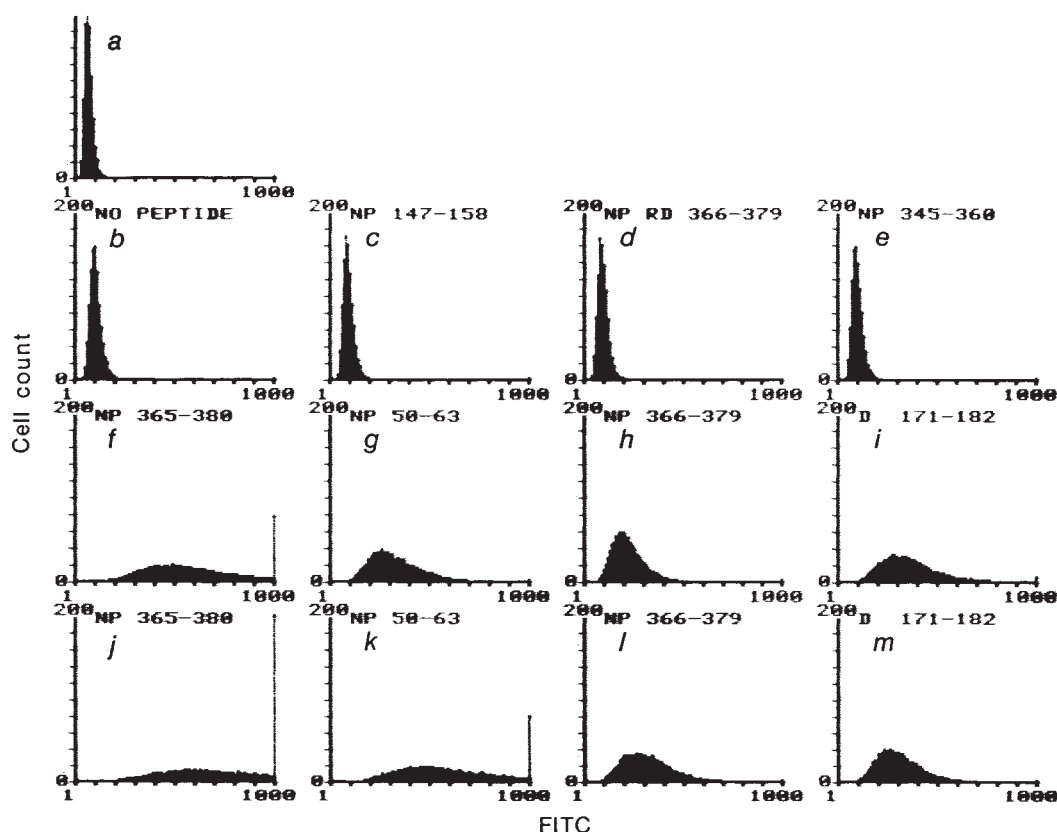


FIG. 1 CTL clone F5 recognizes RMA-S cells treated with peptide NP(1968) 366-379, but not infected with influenza A virus. *a*, Target cells were: ■, RMA infected with influenza E61-13-H17; □, RMA uninfected; ▲, RMA-S infected with E61-13-H17; △, RMA-S uninfected. *b*, Target cells were: ■, RMA treated with NP(1968) 366-379; □, RMA untreated; ▲, RMA-S treated with NP(1968) 366-379; △, RMA-S untreated.

METHODS. Clone F5 was isolated from an influenza infected C57BL/6 mouse as described². Its activity was tested by ⁵¹Cr release assay on influenza E61-13-H17 virus-infected or peptide-treated (5×10^{-5} M for 1 h) RMA-S and RMA cells as described³. [⁵¹Cr]-labelled target cells (2×10^4) were exposed to varying numbers of CTL clone F5 cells to give the ratios of killer to target (K:T) shown. After 4 h of contact the ⁵¹Cr released from target cells into the supernatant was measured and per cent specific lysis calculated as: (release by CTL - release in medium alone)/(2.5% triton X-100 release - release in medium alone). Spontaneous ⁵¹Cr released in medium was 12-19% of that released by triton X-100.

FIG. 2 The indirect immunofluorescence staining of RMA-S cells exposed to various peptides for 5 or 24 h. *a*, Background staining with no first antibody (NFA); *b*, Low level B22.249 (D^b) specific staining detected on untreated RMA-S cells. The remaining panels show RMA-S cells stained with B22.249 after treatment with various peptides: *c*, NP(1968) 147–158 for 5 h; *d*, retro-D isomer of NP(1968) 366–379 for 5 h; *e*, NP(1968) 345–360 for 5 h; *f*, NP(1934) 365–380 for 5 h; *g*, NP(1968) 50–63 for 5 h; *h*, NP(1968) 366–379 for 5 h; *i*, H-2 D^b residues 171–182 (ref. 19) for 5 h; *j*, NP(1934) 365–380 for 24 h; *k*, NP(1968) 50–63 for 24 h; *l*, NP(1968) 366–379 for 24 h; *m*, H-2 D^b 171–182 for 24 h.

METHODS. RMA-S cells (5×10^5) were exposed to medium alone or peptides at 5×10^{-5} M in a total volume of 1.5 ml for the times stated. Cells were then collected and stained by indirect immunofluorescence as described². The first layer was the D^b specific monoclonal antibody B22.249 (ref. 45) as neat culture supernatant (C/S), and the second layer



was FITC-labelled affinity purified goat anti-mouse antibody (Sigma) at 1:40 dilution. The samples were analysed on an Ortho Cytofluorograf.

(amino acids 366–379 of NP (A/NT/60/68³). We found that the two cells were recognized to the same extent after exposure to peptide at concentrations $> 10^{-6}$ M (Fig. 1*b*), despite the fact that untreated RMA-S cells normally express $\sim 1/20$ the number of H-2 D^b molecules as RMA. These results led us to speculate that treatment of RMA-S with the peptide may have increased expression of H-2 D^b at the cell surface.

Cell surface expression of class I

A comparison of RMA-S before and after exposure to NP (1968) 366–379, or to the related sequence NP(1934) 365–380, revealed

an increase in D^b expression of between two- and fivefold (Fig. 2*f*, *h*, *j* and *l*). This was detected with an antibody demonstrated to bind only to heavy chains associated with β_2 -microglobulin²².

We then tested two additional peptides that prevent presentation of the sequence NP(1968) 366–379 to the CTL clone F5¹⁹. The inhibitory effect of these peptides indicates that they bind the D^b molecule¹⁹ although this has not been demonstrated directly. One peptide was derived from the NP sequence (residues 50–63). The other is from a conserved region of the D^b molecule itself (residues 171–182). We chose the latter because of its homology with the equivalent sequence from

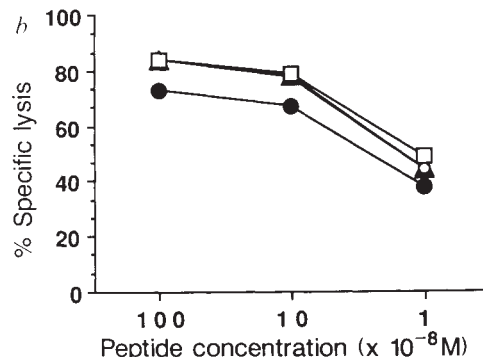
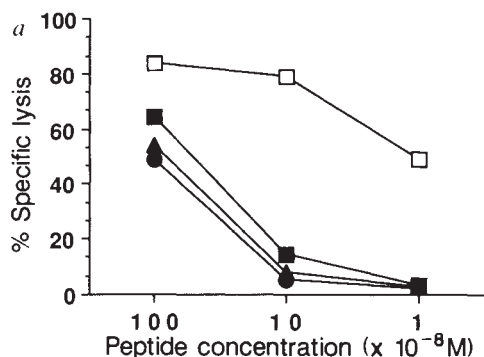
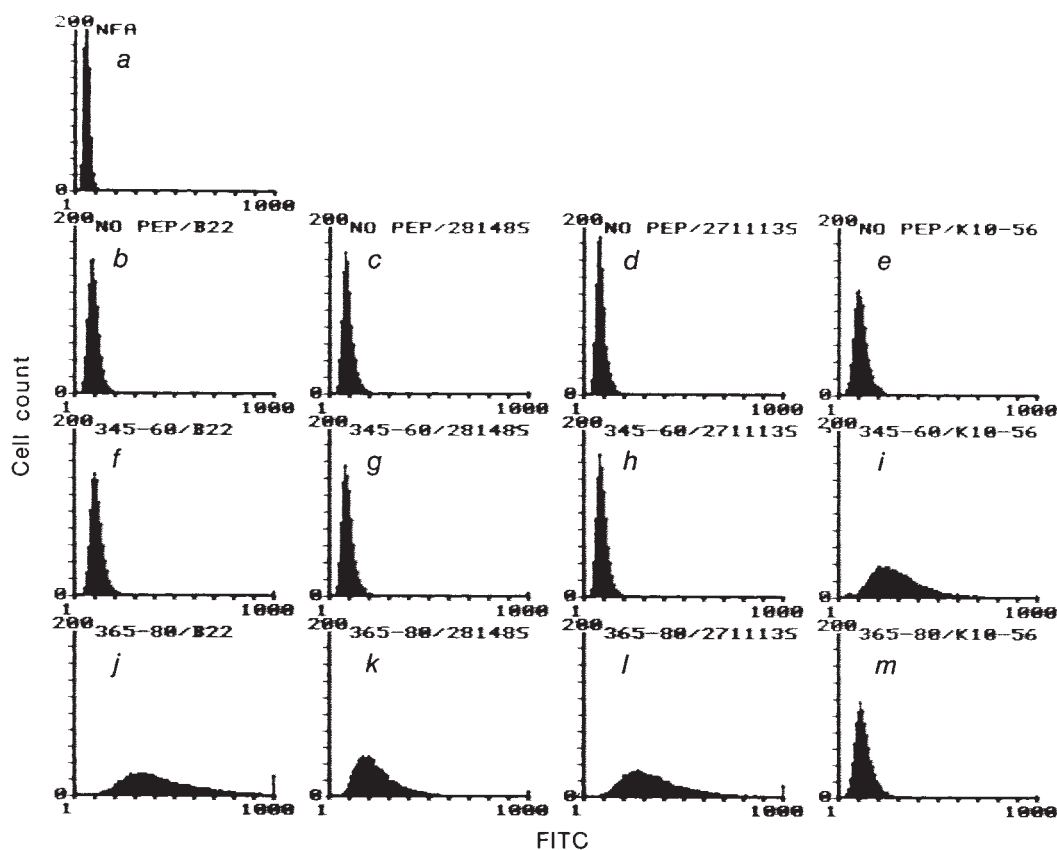


FIG. 3 Recognition by clone F5 of RMA-S cells exposed to NP(1968) 366–379 is inhibited by defined competitor peptides. *a*, Three sequences that compete efficiently: ■, NP(1934) 365–380; ▲, NP(1968) 50–63; ●, H-2 D^b 171–182; □, no competitor. *b*, Three sequences that do not compete: ▲, retro-D isomer of NP(1968) 366–379; ●, NP(1968) 147–158; ○, NP(1968) 345–360; □, no competitor.

METHODS. [^{51}Cr]-labelled RMA-S cells were exposed to the peptide NP(1968) 366–379 at the concentrations shown either alone, or in combination with

competitor peptides at a fixed concentration of 10^{-4} M. The ratios of competitors to NP(1968) 366–379 therefore ranged between $10^4:1$ to $10^2:1$. The competitor sequences were found by trial and error as described¹⁹ and were derived from published influenza NP sequences³ except for the peptide comprised of residues 171–182 of H-2 D^b which was chosen on the basis of its relationship to the homologous sequence in HLA Cw3, which has similar inhibitory activity^{19–21}.

FIG. 4 Induction of class I molecules on RMA-S cells by peptides is H-2 allele specific. *a*, Background staining with no first layer antibody (NFA). *b-e*, Untreated RMA-S cells stained with the following antibodies: *b*, B22.249 specific for the $\alpha 1$ domain of H-2 D^b (refs 22-24); *c*, 28148S specific for the $\alpha 3$ domain of H-2 D^b (refs 22, 23); *d*, 271113S specific for the $\alpha 1$ domain of H-2 D^b (refs 22-24); *e*, K10-56 specific for the $\alpha 1 + \alpha 2$ domains of H-2 K^b (refs 23, 46). *f-i*, RMA-S cells treated with 10^{-4} M NP(1968) 345-360 for 6 h and stained with: *f*, B22.249 ($\alpha 1$, D^b); *g*, 28148S ($\alpha 3$, D^b); *h*, 271113S ($\alpha 1$, D^b); *i*, K10-56 ($\alpha 1 + \alpha 2$, K^b). *j-m*, RMA-S cells treated with 10^{-4} M NP(1934) 365-380 for 6 h and stained with: *j*, B22.249 ($\alpha 1$, D^b); *k*, 28148S ($\alpha 3$, D^b); *l*, 271113S ($\alpha 1$, D^b); *m*, K10-56 ($\alpha 1 + \alpha 2$, K^b). METHODS. RMA-S cells were exposed to peptides NP(1934) 365-380 or NP(1968) 345-360 at 10^{-4} M for 6 h and stained by indirect immunofluorescence as described in Fig. 2. The first layer antibodies were either neat culture supernatants (B22.249, K10-56) or



ascites purified on protein A Sepharose (28148S, 271113S) as described⁴⁷ and used at $5 \mu\text{g m}^{-1}$ (ref. 2).

HLA Cw3, which has a comparable inhibitory activity¹⁹⁻²¹. To ensure that the D^b molecules on RMA-S (which had been exposed to a mutagen) retained their specificity for peptides, the inhibitory effect of these peptides was assayed using RMA-S cells (Fig. 3*a* and *b*).

The peptides that inhibited the recognition of RMA-S by the D^b restricted clone F5 also induced expression of D^b at the surface of RMA-S cells (Fig. 2*f*, *g* and *i*). The three control peptides did not inhibit recognition by clone F5, nor did they induce expression of D^b (Fig. 2*c*, *d* and *e*). There is therefore a correlation between induction of D^b expression on RMA-S

and the ability to inhibit presentation of NP(1968) 366-379 in the lysis assay, implying that only peptides that bind D^b induce its expression.

The class I molecule D^b is unusual because it can reach the cell surface in the absence of endogenous β_2 -microglobulin synthesis in the murine cell line R1E (ref. 22). Most class I antibodies bind MHC class I heavy chains only when they associate with β_2 -microglobulin but free D^b heavy chains can be detected with the antibody 28148S, which binds the $\alpha 3$ domain of both free and β_2 -microglobulin-associated heavy chains²³. Free D^b heavy chains were not detected by an excess

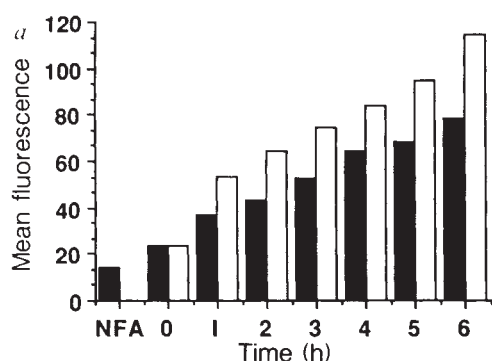
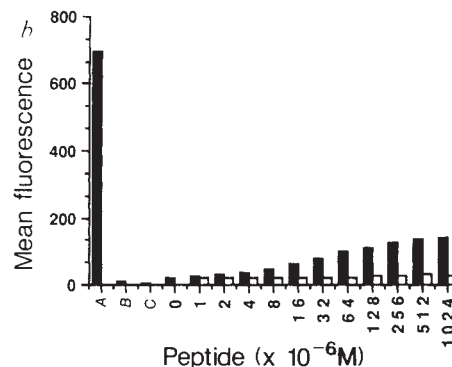


FIG. 5 Time course (*a*) and dose response (*b*) of the induction of H-2 D^b on RMA-S by peptide. *a*, Time course in response to 2.5×10^{-4} M ($\square$); or 0.5×10^{-4} M ($\blacksquare$) of NP(1934) 365-380. NFA, background staining with no first layer antibody. *b*, Dose response of the induction of D^b on RMA-S by peptides NP(1934) 365-380 ($\blacksquare$); and NP(1968) 345-360 ($\square$). A, RMA stained with B22.249 for comparison; B, background staining of RMA with no first antibody; 0, untreated RMA-S stained with B22.249.



METHODS. Aliquots of 5×10^5 RMA-S cells in 0.75 ml medium (RPMI 1640/10% FCS) were placed in Costar 24-well plates. *a*, At times 0, 1, 2, 3, 4 and 5 h 0.75 ml of pre-warmed medium containing twice the desired concentration of peptide was added to appropriate aliquots of cells. At 6 h all the cells were collected and stained by indirect immunofluorescence with antibody B22.249 as described in Fig. 2. *b*, All aliquots of cells were incubated with peptide for 6 h at 37 °C. The final concentrations of peptides ranged from $1-1,024 \times 10^{-6}$ M as shown.

of 28148S binding on the surface of RMA-S cells, either before or after induction with peptides (Fig. 4c and k).

We used additional class I-specific monoclonal antibodies to show that the effect of peptides on RMA-S is H-2 allele-specific (Fig. 4). Treatment with NP(1934) 365-380 induced D^b (compare Fig. 4a-d with 4j-l), but not K^b (Fig. 4e and m). In contrast, NP(1968) 345-360 has the opposite effect, inducing K^b (Fig. 4i) but not D^b (Fig. 4f-h). The polyclonal cytotoxic T-cell response to influenza nucleoprotein in H-2^b mice is not restricted through K^b (ref. 5). The induction of K^b by a peptide is not unexpected, however, as we have found other peptides which induce D^b on RMA-S cells (Fig. 2), and compete in the CTL lysis assay (Fig. 3), but which are not epitopes recognized by CTL from H-2^b mice¹⁹.

The time course and dose response characteristics of the induction of H-2D^b on RMA-S are shown in Fig. 5a, b. The effect is detected after one hour of exposure to peptide, and increases over six hours. The maximum level of D^b induced was ~1/5 of that found on the RMA cell.

Association of D^b with β_2 -microglobulin

The time taken to double the number of D^b molecules on RMA-S cells treated with peptides was between 1 and 3 hours, depending on the concentration of peptide (Fig. 5a). The speed of this effect indicated that peptides could be inducing transport of intracellular D^b molecules to the cell surface, which might occur should peptides induce the association of D^b heavy chains with endogenous β_2 -microglobulin.

RMA-S cells were therefore labelled with [³⁵S]methionine for 40 minutes after exposure to peptides, and the D^b heavy chains (both free and β_2 -microglobulin associated) were immunoprecipitated with the antibody 28148S which is specific for the $\alpha 3$ domain²²⁻²⁴. If association with endogenous β_2 -microglobulin is stimulated by peptides, then more β_2 -microglobulin should be co-precipitated with D^b heavy chains.

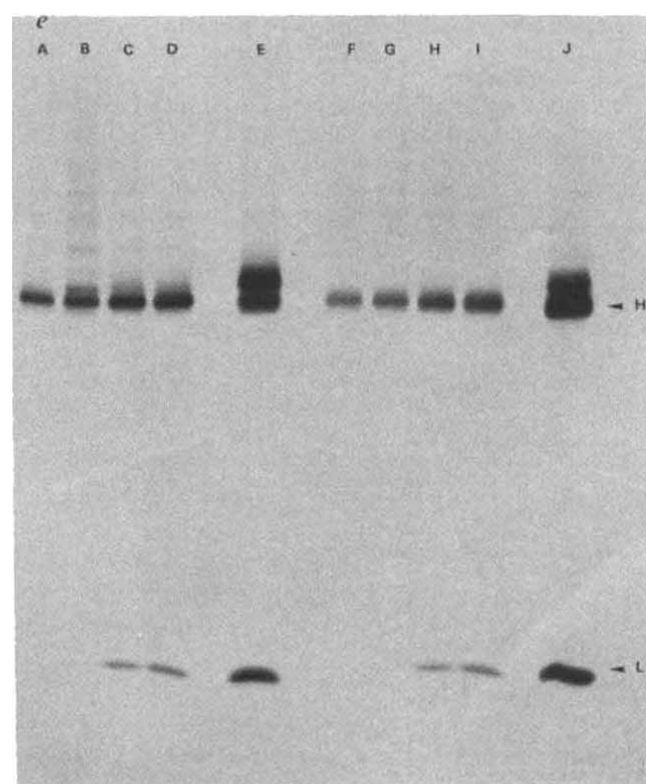
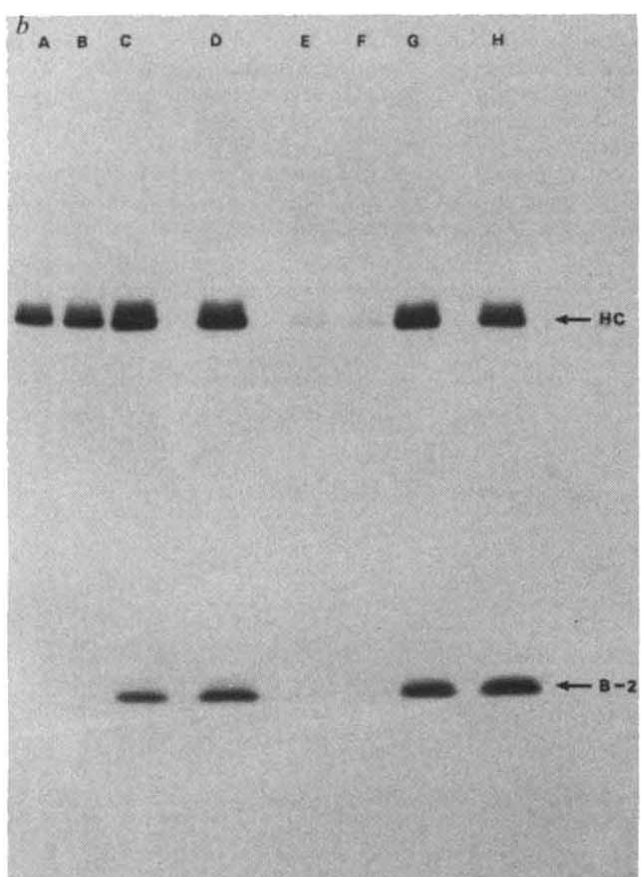
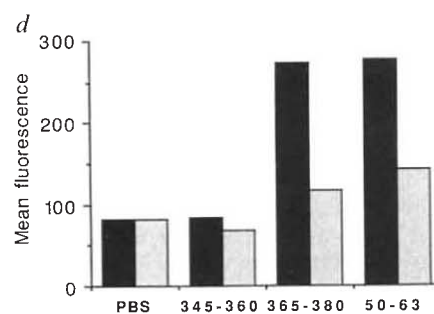
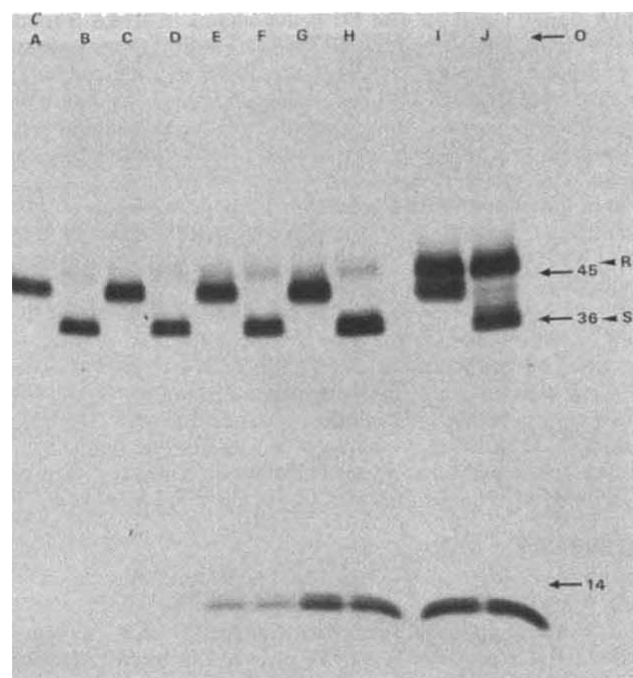
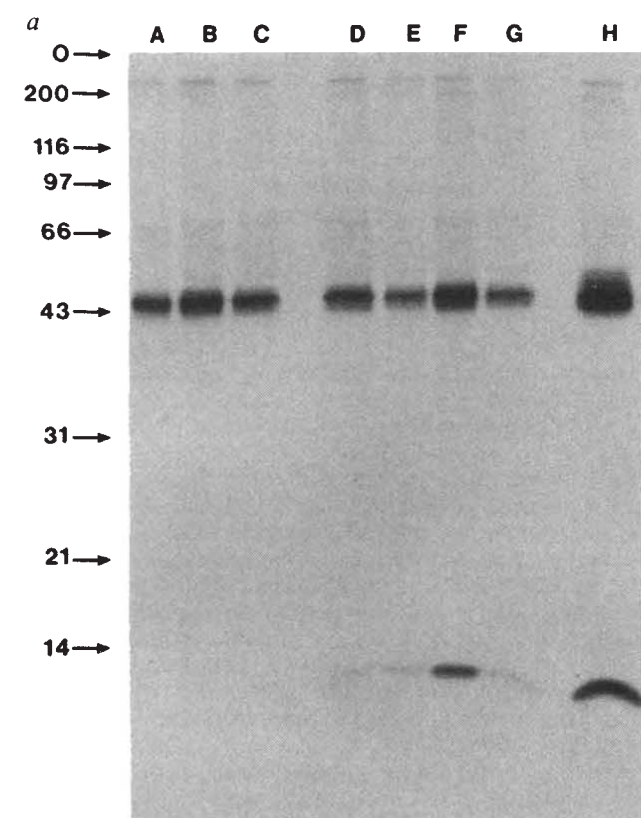
Figure 6a, lane A, shows that D^b heavy chains in RMA-S co-precipitated a barely detectable amount of β_2 -microglobulin¹⁸. Lanes B and C show that treatment with two control peptides had no effect on chain association. In contrast, each of the four peptides thought to bind D^b increased the amount of co-precipitated β_2 -microglobulin (lanes D-G and H, respectively). Of these, NP(1968) 50-63 (lane F) seemed to be the most effective. This correlates with the observation that NP(1968) 50-63 is a more potent inhibitor of recognition by D^b-restricted T-cells¹⁹ and so may have a higher affinity for D^b.

Not only assembly, but folding of the D^b heavy chain is driven by peptide (Fig. 6b). The antibody B22.249 reacts with a determinant on the $\alpha 1$ domain of D^b that appears only when D^b is associated with β_2 -microglobulin²². Lanes A and B of Fig. 6b define a cohort of D^b molecules that were labelled in RMA-S during a 15-min pulse with [³⁵S]methionine. The majority of these were neither associated with β_2 -microglobulin (lanes A and B), nor reactive with the $\alpha 1$ domain specific antibody B22.249 (lanes E and F). However, in cells exposed to NP(1968) 50-63, the D^b heavy chains became associated with β_2 and folded into a conformation detected by B22.249, within 15 minutes of synthesis (compare lanes A, B, C with E, F, G). We have also noted that the antibody 28148S (specific for the $\alpha 3$ domain²²⁻²⁴), precipitates D^b heavy chains more efficiently when they are associated with β_2 -microglobulin (compare lane A with C). It may therefore bind to assembled class I molecules with higher affinity than to free heavy chains.

The state of glycosylation of the heavy chains in peptide-treated cells is shown in Fig. 6c. RMA-S cells were labelled for 5 hours in the presence of peptides, to allow accumulation of heavy chains that have acquired endo H resistance on passing through the Golgi (refs 25-28). Lanes A-D show that in untreated RMA-S and cells treated with a control peptide, D^b heavy chains are associated with a low level of β_2 -microglobulin and acquire minimal endo-H resistance compared with those in

FIG. 6 Effect of peptide on assembly with β_2 -microglobulin and movement through the Golgi. a, Co-precipitation of β_2 -microglobulin with D^b heavy chains from RMA-S treated with peptides for 6 h and labelled for 40 min. Lane A, untreated RMA-S; B, RMA-S + NP(1968) 345-360; C, RMA-S + retro-D isomer NP(1968) 366-379; D, RMA-S + NP(1968) 366-379; E, RMA-S + NP(1934) 365-380; F, RMA-S + NP(1968) 50-63; G, RMA-S + H-2 D^b 171-182; H, RMA untreated. b, Peptide induces a conformational change in the $\alpha 1$ domain of the D^b heavy chain in RMA-S cells. The samples in lanes A-D were immunoprecipitated with the antibody 28148S (specific for the $\alpha 3$ domain), and those in lanes E-H with B22.249 (specific for the $\alpha 1$ domain). Lanes A & E, RMA-S untreated; B & F, RMA-S + NP(1968) 345-360; C & G, RMA-S + NP(1968) 50-63; D & H, RMA untreated. c, D^b heavy chains in RMA-S acquire Endo H resistance after treatment with peptides. The samples were immunoprecipitated with 28148S and in lanes A, C, E, G, I were mock digested, and in B, D, F, H, J digested with Endo H. The positions, after digestion, of resistant (R) and sensitive (S) heavy chains are marked to the right of the figure. Lanes A, B, RMA-S + no peptide; C, D, RMA-S + NP(1968) 345-360; E, F, RMA-S + NP(1934) 368-380; G, H, RMA-S + NP(1968) 50-63; I, J, RMA + no peptide. d, Brefeldin A blocks the peptide-induced expression of D^b at the cell surface. RMA-S cells were treated with 1 μ g ml⁻¹ BFA (■), or mock-treated (□), and exposed to the peptides shown for 5 h. D^b expression was measured with the antibody B22.249. e, BFA does not inhibit class I assembly induced with peptides. Lanes A-E, immunoprecipitates from mock treated cells; F-J, immunoprecipitates from BFA-treated cells. A & F, RMA-S + no peptide; B & G, RMA-S + NP(1968) 345-360; C & H, RMA-S + NP(1934) 365-380; D & I, RMA-S + NP(1968) 50-63; E & J, RMA control + no peptide.

METHODS. a, RMA-S and RMA cells were resuspended at 10⁶ ml⁻¹ in medium (RPMI 1640/10% FCS) alone or containing peptides at 10⁻⁴ M for 5 h. They were then washed twice in warmed phosphate buffered saline (PBS) and resuspended at 5 × 10⁶ ml⁻¹ in methionine-free medium containing peptides at the same concentration for a further hour. [³⁵S]-methionine (120 μ Ci) was added and the mixture incubated for 40 min at 37 °C. The labelled cells were then washed once in ice-cold PBS and resuspended in 0.5 ml lysis buffer (0.5% v/v NP40, 0.5% mega 9 (ref. 48), 150 mM NaCl, 5 mM EDTA, 50 mM TRIS pH 7.5) containing 2 mM PMSF and 5 mM iodoacetamide. The lysates were precleared with *Staphylococcus A* organisms overnight at 4 °C and immunoprecipitation was performed as described² with purified 28148S antibody at a final concentration of 5 μ g ml⁻¹. Reduced immunoprecipitates were electrophoresed on a 12% SDS polyacrylamide gel, which was fixed, stained, treated with Amplify (Amersham), dried and exposed to pre-flashed X-ray film. b RMA-S and RMA cells were resuspended at 5 × 10⁶ ml⁻¹ in medium alone or containing peptides at 2 × 10⁻⁴ M for 4 hours. Aliquots of cells (2 × 10⁷) were then washed and resuspended in 1 ml methionine free medium containing peptides at the same concentration for one hour. [³⁵S]-methionine (250 μ Ci) was then added and the mixtures incubated at 37 °C for 15 minutes. The cells were then washed once in 15 ml of ice cold PBS containing 2 mM of unlabelled L-methionine and lysed as described above. The lysates were divided into two equal parts, either 28148S or B22.249 added to 10 μ g ml⁻¹, and immunoprecipitates prepared as described above. c, Aliquots of 1.8 × 10⁷ RMA-S or RMA cells were resuspended in 1 ml of methionine-free medium alone, or containing peptides at 2 × 10⁻⁴ M. After incubation for 1 h, 250 μ Ci [³⁵S]-methionine was added and the mixtures incubated for 5 h at 37 °C. The cells were lysed in 1 ml lysis buffer (as above), and immunoprecipitates prepared with the 28148S antibody at 10 μ g ml⁻¹. These were divided into two equal portions. One was digested with 0.005 U ENDO H (Boehringer) as described²⁵, the other was mock-digested. The samples were trichloroacetic acid precipitated with 50 μ g Sigma SDS-7 molecular weight markers as carrier, then analysed as in (a). d, RMA-S cells (5 × 10⁶) were aliquotted in 0.9 ml of medium. BFA (2 μ l, 0.5 mg ml⁻¹ in methanol), or methanol, were added. (In control experiments we established that methanol at this dilution had no effect on class I expression.) After 45 min incubation at 37 °C, peptides (100 μ l in PBS at 1 mM), or PBS, were added and the mixtures incubated at 37 °C for 5 h. The cells were then collected and stained by indirect immunofluorescence with the antibody B22.249 as described in Fig. 2. e, In parallel with experiment (d), aliquots of 5 × 10⁶ RMA-S or RMA cells were resuspended in 0.9 ml methionine-free medium, and BFA (2 μ l, final concentration 1 μ g ml⁻¹) or methanol were added as above. After 45 min peptides were added to 10⁻⁴ M as above, followed by 100 μ Ci of [³⁵S]-methionine. The mixtures were incubated for 5 h at 37 °C. Immunoprecipitates were prepared and analysed as in (a).



RMA (lanes I and J). The D^b heavy chains in RMA-S treated with the two active peptides (lanes E-H) bound more β_2 -microglobulin, and acquired a degree of endo-H resistance comparable to the level of D^b induced at the cell surface (compare with Fig. 5b). On repeating this experiment with a polyclonal serum to immunoprecipitate heavy chains¹⁸, we obtained the same result (data not shown).

As another test of the induction by peptides of new class I molecules at the cell surface, we investigated the effect of brefeldin A (BFA). In cells treated with BFA, movement of newly synthesized membrane proteins from the endoplasmic reticulum (ER) to the Golgi apparatus is blocked²⁹⁻³², but endocytosis and protein synthesis are not inhibited²⁹. BFA at 1 $\mu\text{g ml}^{-1}$ blocked the induction of D^b at the RMA-S cell surface by 69-92% in three trials. The same concentration of BFA did not, however, inhibit β_2 -microglobulin association with D^b (Fig. 6d and e). This indicates that peptides in BFA-treated cells can still enter the cell and induce chain association, but transport of assembled class I molecules out of the ER is blocked.

Discussion

We have shown that exposure of the RMA-S mutant cell to certain peptides partially restores association of D^b heavy chains with β_2 -microglobulin, and expression of D^b or K^b molecules at the cell surface (Fig. 2, 4). We have also observed that both RMA and L cell fibroblasts, or the NS-0 myeloma transfected with the D^b gene, respond with a modest increase in class I expression (33-235%) after exposure to appropriate peptides (data not shown). Normal cells might therefore exhibit a less exaggerated form of the same phenomenon.

The evidence in Fig. 6 favours the interpretation that peptides at high concentration in extracellular fluid can reach a pre-Golgi compartment of RMA-S cells, where they induce the D^b heavy chain to fold and associate with β_2 -microglobulin. This compartment could be the ER, or possibly an intermediate compartment between the ER and the Golgi³³. The assembled complexes are then transported to the cell surface. The peptide NP(1968) 50-63 appears to induce chain association more effectively than NP(1934) 365-380 (Figs 6a, b and c), but induces the equivalent or slightly less D^b at the cell surface (Figs 2 and 6c). The assembled D^b complexes formed by these two peptides may therefore differ in their stability, or rate of transport to the surface of the cell.

The effect on D^b is specific to peptides identified either as epitopes recognized in association with D^b by CTL (refs 3, 7),

or as sequences able to prevent the presentation of known epitopes to D^b-restricted CTL (ref. 19), implying that only peptides binding D^b or K^b increase their expression at the surface of RMA-S cells.

The most probable site for peptide binding is the groove formed by the polymorphic $\alpha 1$ and $\alpha 2$ domains of the class I heavy chain³⁴. Deletion of these domains prevents binding of β_2 -microglobulin to the $\alpha 3$ domain of the D^b molecule²². Furthermore, in the crystal structure of HLA-A2, β_2 -microglobulin makes multiple contacts with the $\alpha 1$ and $\alpha 2$ domains³⁵, but almost certainly would not make direct contact with bound peptide. Chain association may therefore depend on correct folding of these domains. Our results indicate that folding may depend on the availability of peptide ligands. The $\alpha 1$ and $\alpha 2$ domains of newly synthesized class I heavy chains could fold around the peptide to form a three-dimensional structure with affinity for β_2 -microglobulin.

This concept does not rule out the exchange of one bound peptide for another after the class I molecule has assembled and is exposed at the cell surface. Peptide exchange is no less conceivable than β_2 exchange³⁶. The observations that fixed cells³⁷ and cells treated with BFA (ref. 32) can present extracellular peptide to class I-restricted T-cells, implies that peptide exchange can occur at the surface of non-mutant cells. But the extremely low level of saturation (0.3%) of class I achieved with peptides *in vitro*, argues that it is inefficient³⁸.

Our hypothesis could explain why class I heavy and light chains synthesized *in vitro* in the absence of peptide fail to associate³⁹, and also the nature of the mutations that could have given rise to the RMA-S cell and others resembling it⁴⁰⁻⁴². Mutations in the heavy chain, or β_2 -microglobulin, could interfere with class I assembly^{43,44}. This is unlikely in RMA-S, however, because fusion with L cell fibroblasts restores class I assembly, expression, and antigen presentation (C.O. *et al.*, manuscript in preparation).

On the other hand, if assembly of class I molecules depends on peptide binding, a defect reducing the concentration of peptides during folding would prevent chain association. BFA blocks presentation of a cytoplasmic antigen to class I-restricted T-cells, suggesting that peptides derived from cytoplasmic proteins in normal cells may be transported into a pre-Golgi compartment³². Proteins without hydrophobic signal sequences are efficiently presented^{2,6}. Entry of peptides into this compartment may therefore involve a specialized mechanism, loss of which could account for the phenotype of RMA-S cells. □

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MERLIN observations of the Wolf-Rayet star AS431 (WR147)—a double radio source

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THE Wolf-Rayet star AS431 (number 147 in ref. 1) is known to be bright at X-ray and radio wavelengths^{2,3}, and previous radio-interferometric observations indicate that it has an extended structure³. Here we report new interferometric observations of AS431 using MERLIN (the Jodrell Multi-Element Radio Linked Interferometer Network), in which the sensitivity was sufficient to map the stellar thermal-continuum radio emission with a beam size of 0.1 arcsec. The object is resolved into two radio sources, separated by 0.6 arcsec, corresponding to a spatial separation of 1,100 AU at the assumed distance of 1.9 kpc. The computed brightness of the two sources, $\sim 10,000$ K, is typical of optically thick thermal emission from a stellar wind, but compared with other observations² suggests that some part of the emission is non-thermal. One of the radio components corresponds to the position of the single source revealed by accurate optical observations. We simulate the observed total radio spectrum with a two-component model for the emission.

AS431 was observed on 30 January 1988 at 5 GHz with a bandwidth of 9 MHz for a total period of 15 h. As part of the MERLIN extension project, the 5 GHz receivers have recently

been equipped with cooling so that the system temperatures on the individual telescopes ranged from 30 K to 40 K. Data analysis was performed on the Starlink node in conjunction with the Alliant FX/8 computer at Jodrell Bank.

As the expected radio flux density was low, a phase-referencing technique⁴ was used in which 12-min observations of AS431 were interspersed with 3-min observations of a phase reference source. The data were edited, vector averaged and calibrated using routines in the OLAF (off-line analysis format—an astronomical image processing package designed specifically to process MERLIN or VLBI data) software package; the visibility data were transformed to an intensity map using the astronomical image processing system (AIPS). First, the phase reference source was mapped by the standard self-calibration technique⁵. The derived telescope gain solutions were then applied to AS431 and the resulting data further processed by Fourier and CLEAN techniques⁶. Using these techniques, the flux density and position of the source were accurately related to that of the phase reference. The flux density of the reference source, 2055+403, was determined to be 4.4 Jy using a comparison observation of the calibration source 3C286, the flux density of which was assumed to be 7.4 Jy at 5 GHz.

The resulting map at 5 GHz with a beam of $0.15 \times 0.13''$ is shown in Fig. 1. The source is clearly resolved into two components, separated by ~ 0.6 arcsec. Table 1 gives details of the positions and flux densities of the two components. Both are more extended than the beam size and the table gives the gaussian sizes and calculated brightness temperatures, T_b .

Previous radio observations of the source with the Very Large Array (VLA) in its more compact (C and D) configurations^{2,3} have found the source to have a total flux density of 35 mJy at 5 GHz. Abbott *et al.*³ concluded that the source was extended from their 1984 VLA C array observations, and fitted a single-component wind model with temperature 6,000 K to the visibilities. The radio spectrum between 1.5 and 5 GHz was essentially flat², which is contrary to that expected from an optically thick bremsstrahlung source, and this led to the suggestion that the source may contain both thermal and non-thermal components to its radio emission.

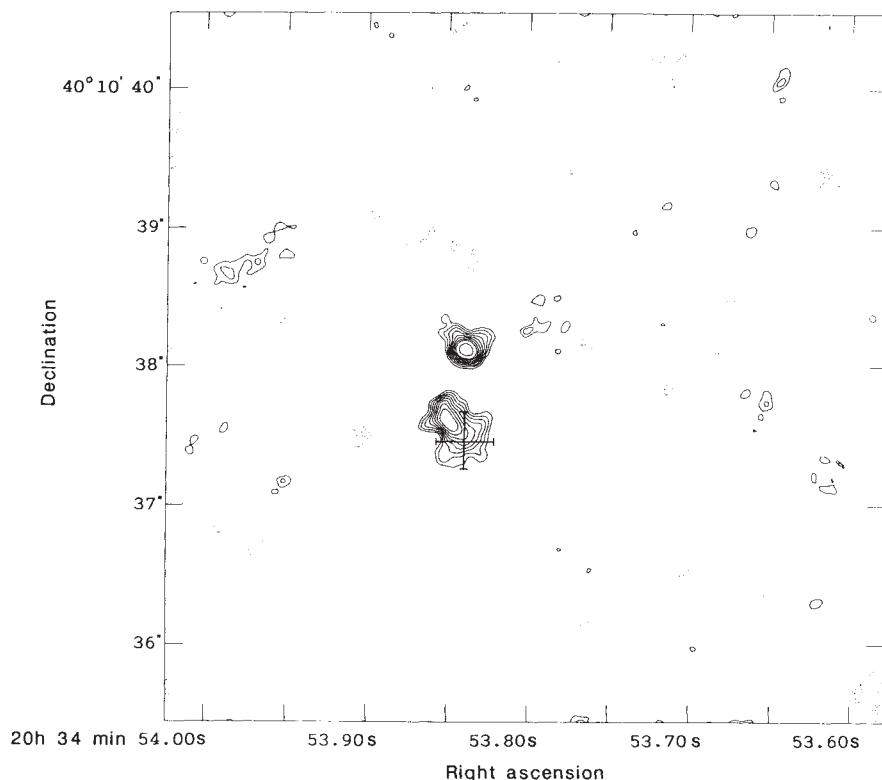


FIG. 1 MERLIN 5 GHz map of AS431 on 30 January 1988 compared with the measured position of the optical source. The peak flux density per beam in the radio map was 4.2 mJy and a restoring beam of $0.15 \times 0.13''$ at a position angle of -37° was used. The r.m.s. noise was 0.36 mJy. Radio contours are at 0.3 mJy intervals; the bottom contour is 0.9 mJy; negative contours are dashed. The mean optical position with 1σ error bars is also plotted.

TABLE 1. AS431 details

	Position					Flux	Gaussian	Position	Bright-	
	RA		1950		dec	density	size	angle	ness	
	h	min	s	deg	min	s	(mJy)	(arcsec)	temperature	
								(major	T_b (K)	
								axis deg)		
N	20	34	53.836	+40	10	38.130	10.8	0.20 × 0.18	61	16,700
S	20	34	53.850	+40	10	37.613	21.8	0.42 × 0.29	36	9,900

Although our data do show that there is more than one component, non-thermal emission need not be invoked for either source if we take our results in isolation. The inferred brightness temperatures are almost canonical for thermally emitting optically thick circumstellar gas ($\sim 10,000$ K). The almost flat radio spectrum is problematic, however, suggesting that the sources are either optically thin thermal bremsstrahlung (with an electron temperature at least an order of magnitude higher than the T_b measured here) or a combination of thermal and non-thermal emission³. As some Wolf-Rayet stars are known to be variable at radio wavelengths it is perhaps premature to assume that the spectrum is still flat but there is little evidence of variability in this source.

Optical CCD (charge-coupled device) images of AS431 were obtained as service observations on the 4.2-m William Herschel Telescope (WHT) on La Palma on 18 September 1988 through narrow-band H α and broad-band R filters under good (0.6") seeing. Although the source was apparently extended in these images, comparison with concurrent observations of a reference star (SAO49845) confirmed that the elongations present were due to altitude and azimuth tracking irregularities and that the images were compatible with a single optical source. No secondary source was evident within 0.6" north or south of the source with $\Delta m < 1.9$ mag, confirming the optical-speckle results⁷.

To determine the relation between this single optical source and the radio map, two photographs of the field were taken with Cambridge University's f3.8 40/60 cm classical Schmidt telescope using 10.2 × 12.7 cm Kodak T-max film on the nights of November 2 and 4 1988. These films were measured using the Cambridge automatic plate measuring machine (APM) and the optical position determined by reference to 35 stars in the Heidelberg PPM catalogue⁸. The resulting positions from the two films were: 2 November, $\alpha = 20$ h 34 min 53.85 s, $\delta = +40^\circ 10' 37.6''$; 4 November, $\alpha = 20$ h 34 min 53.83 s, $\delta = +40^\circ 10' 37.3''$ (equinox 1950, epoch 1988.9). The standard error on the mean of these two positions is 0.22". The systematic uncertainty between the VLA calibrator position (on which the MERLIN radio positions are based) and the PPM result is around 0.1". The mean position is plotted in Fig. 1. The optical stellar source coincides with the southern radio object, being 3σ from the northerly source and 1.9σ from the midpoint of the two.

From Table 1 it can be seen that the southerly source is more extended, with lower T_b than the northerly component; it also seems to coincide with the optically bright stellar source. We therefore suggest that the southernmost radio source originates

from thermal emission from a stellar wind. Assuming that this is the source of all the thermal emission in AS431, the spectral index from 5 GHz to 25 μ m (the latter being from the IRAS point source catalogue with stellar subtracted—ref. 9) would be $\alpha = 0.6$ ($s = \nu^{+\alpha}$, where s is the flux density and ν is the frequency). This is the canonical value for thermal bremsstrahlung emission from a constant-outflow optically thick ionized wind¹⁰.

Using equation (20) of ref. 10, we may then calculate the required mass loss rate for such a process from this component, assuming an optical depth of unity and a uniform isotropic flow

$$\dot{M} = CV_i S_\nu^{3/4} D^{3/2} M_\odot \text{ yr}^{-1}$$

where V_i is the terminal velocity in the wind in km s⁻¹, S_ν the observed flux density in Jy, D the distance in kpc and C a constant which is sensitive only to ionic composition of the wind. For Wolf-Rayet stars it seems that C is between 0.7×10^{-6} and 2.1×10^{-6} (ref. 3). Taking $C = 1.4 \times 10^{-6}$, $D = 1.9$ kpc and $V_i = 1,000$ km s⁻¹ (ref. 3) and a flux density of 21.4 mJy, we calculate a mass loss rate of $2.1 \times 10^{-4} M_\odot \text{ yr}^{-1}$. This is still several times greater than typical mass loss rates calculated for single Wolf-Rayet stars³. It should be noted however that $\dot{M}$ is very sensitive to D and the quoted distance is uncertain by ± 1 kpc.

At 1.5 GHz the thermal contribution to the radio flux would then be 10.4 mJy. Assuming that the total 1.5-GHz emission is constant at 35 mJy, this leaves an additional component with flux density 24.6 mJy. This corresponds to a calculated spectral index for the northerly source in the 5 GHz map of $\alpha = -0.7$, which is typical for a non-thermal source. Apparently we have a southerly source with strong emission from an ionized wind with a large mass flux and a northerly source which may be the site of optically thin non-thermal emission.

In the Wolf-Rayet star WR140 (HD193793), periodic non-thermal emission is observed which is associated with the interaction of the wind of the WR with an O star in a highly eccentric 7.9-yr orbit¹¹. If AS431 also has a companion (albeit much fainter optically) then this could be associated with the northerly radio source. As the mass flow rate in the WR wind seems to be constant (from the deduced spectral index) and the orbital period at a minimum separation of 1,100 AU would be several thousand years for reasonable values of WR mass, then it would not be surprising to find that the non-thermal emission does not vary. Finally, we suggest that the same interaction region that gives rise to the non-thermal emission is also the source of the X-ray emission observed in AS431 (ref. 2). The derived gas temperature, in excess of 1.2×10^7 K, is consistent with shock velocities in excess of 700 km s⁻¹. As the mechanical power in the wind of the southerly source is of the order of 3×10^4 times the observed X-ray luminosity, then despite the fact that the solid angle of interaction may be low, and a gas at these temperatures is not a particularly efficient radiator, there is sufficient power available for the X-ray emission.

It would be useful to determine the radio flux at lower frequencies to verify that this emission is dominated by a non-thermal process. $\square$

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Testing nuclear theory using the 0.5 ms pulsar

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IF THE recently reported 0.5-ms optical pulsar in the remnant of SN1987A^{1,2} is indeed a rotating neutron star, then its very existence may be used to rule out many previously viable equations of state of nuclear matter (ref. 3). We demonstrated³ that it may be impossible to spin up an initially non-rotating neutron star to a spin period $P_{\text{rot}} \leq 0.5$ ms for any currently proposed nuclear equation of state. Here, we reverse the argument: a uniformly rotating neutron star with $P_{\text{rot}} \leq 0.5$ ms may be unable to spin down to become a slowly rotating neutron star, if any of the proposed equations of state is correct. Assuming that the neutron star in SN1987A is 'typical' and does not collapse to a black hole as it spins down to $P_{\text{rot}} \gg 0.5$ ms, then its existence may actually invalidate all previously viable nuclear equations of state. We propose an approximate, analytical test that can be used to identify untenable equations of state in the future.

It is always possible to systematically construct families of models of uniformly rotating stars starting from arbitrary non-rotating equilibria³⁻⁷. Of greatest physical relevance are sequences in which the total baryon number is fixed because, as the pulsar spins down, it should not eject an appreciable number of particles. Of course, for a given equation of state, there may be families of models of rotating stars with constant baryon number that do not have non-rotating endpoints⁷. Such equations of state would be viable for the present spin period of the SN1987A remnant, but not for its future, longer, spin period; we discount them.

We first consider an extreme analytic model, the relativistic Roche model, in which the star is assumed to be very centrally condensed. In practice, we shall see that the analytic constraint on non-rotating star models implied by the relativistic Roche model is quite close to numerical constraints derived for a variety of nuclear equations of state⁷.

On the surface of a uniformly rotating star, the metric $g_{\mu\nu}$ satisfies

$$g_{00} + 2g_{0\phi}\Omega + g_{\phi\phi}\Omega^2 = \text{constant} \quad (1)$$

where Ω is the rotation frequency (see, for example, ref. 8, problem 16.19) and ϕ is the longitude. The constant on the right-hand side of equation (1) may be used to (partially) define a family of equilibria. For families with non-rotating endpoints, we may evaluate the constant for the $\Omega = 0$ model. We choose coordinates that reduce to exterior Schwarzschild as $\Omega \rightarrow 0$. Then the constant is just g_{00} for the non-rotating starting point of the sequence. Now we are interested in constructing sequences of fixed rest mass. In the limit of extreme central condensation, stellar masses along such a sequence should remain approximately constant, since rotation does not substantially affect the density profile in the interior, where most of the mass resides. Moreover, in highly concentrated star models, $g_{00} = -(1 - 2M/r)$ and $g_{\phi\phi} = r^2 \sin^2\theta$, where θ is the co-latitude near the surface. We can also ignore $g_{0\phi}$ at the surface, since $g_{0\phi}/(g_{\phi\phi}\Omega) \sim (R_{\text{r.m.s.}}/r)^2$, where $R_{\text{r.m.s.}} (\ll r)$ is the mass-weighted r.m.s. radius of the star⁹. With these approximations, we can use equation (1) to find an equation for the shape $r(\theta)$ of the surface of a rotating star of mass M ,

$$\frac{2M}{r} + \Omega^2 r^2 \sin^2\theta = \frac{2M}{R} \quad (2)$$

where R is the radius of the non-rotating configuration. Now for allowed equilibria, equation (2) must have real solutions for

all θ ; this is only true if³

$$\frac{\Omega}{\sqrt{G\bar{\rho}}} \leq \left(\frac{4\pi}{3}\right)^{1/2} \left(\frac{2}{3}\right)^{3/2} = 1.114 \dots \quad (3a)$$

where $\bar{\rho} \equiv 3M/4\pi R^3$ is the mean density of the non-rotating configuration, or

$$\frac{M}{M_{\odot}} \geq 1.693 \frac{(R/7.5 \text{ km})^3}{(P_{\text{rot}}/0.5 \text{ ms})^2} \quad (3b)$$

In principle, inequalities analogous to equation (3) can be determined numerically for any equation of state. In general, the maximum value of $\Omega/\sqrt{G\bar{\rho}}$ along a given sequence will depend on M/R as well as on precisely what quantity is held fixed in its construction. Rotating star sequences with fixed polar redshift have been calculated for a variety of equations of state; empirically, rest mass is approximately constant along these sequences⁷. Even though the numerical models range over $0.02 \leq M/R \leq 0.3$, we find that $1.11 \leq (\Omega/\sqrt{G\bar{\rho}})_{\text{max}} \leq 1.20$ for all tabulated models (after correcting some apparent inconsistencies in the published parameters of the non-rotating star models)⁷. This range of variation is comparable to the stated accuracy of the numerical results⁷. Thus, $(\Omega/\sqrt{G\bar{\rho}})_{\text{max}}$ is almost independent of M/R at the non-rotating endpoint of all calculated sequences. Moreover, assuming a single maximum rotation rate for all sequences gives, by averaging the numerical values⁷,

$$\frac{\Omega}{\sqrt{G\bar{\rho}}} \leq 1.16 \pm 0.03 \quad (4)$$

which, given the numerical uncertainties, is essentially indistinguishable from equation (3a). The relativistic Roche model provides a surprisingly accurate estimate of the maximum rotation rate along constant rest-mass sequences for many nuclear equations of state.

At the opposite extreme from the relativistic Roche model are models of uniformly dense, incompressible stars. Conservatively, we expect that the maximum rotation rate along constant rest-mass sequences should be bounded by the values for these two extreme density profiles³. There is no simple analytic relation

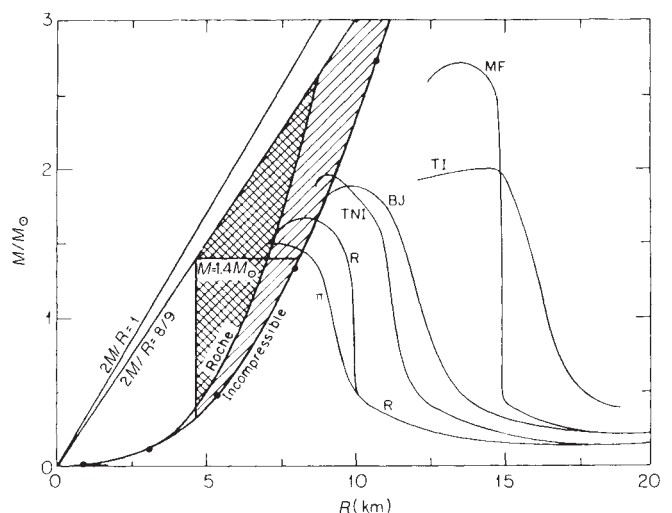


FIG. 1 Non-rotating endpoint equilibrium models for SN1987A. Allowed neutron star configurations must reside in the hatched regions (see text). Models constructed from several nuclear equations of state are shown for comparison: MF, mean field²⁰; TI, tensor interaction²¹; BJ, Bethe and Johnson²²; TNI, three nucleon interaction²³; R=Reid potential²⁴; π , Reid potential with pion condensate¹⁶. Solid dots label incompressible configurations⁶.

like equation (3) for rotating incompressible star sequences in general relativity but Butterworth and Ipser⁴⁻⁶ have constructed sequences for models of incompressible stars with fixed baryon number. We can extract the limiting mass-radius relation for models of incompressible stars from their tabulated numerical results. In the weak field limit, their results⁶ agree with the newtonian (Maclaurin) limit^{3,10} $\Omega/\sqrt{G\rho} \leq 1.188\dots$ for the existence of rotating star equilibria.

Figure 1 summarizes the constraints that can be derived for non-rotating star models if we assume that the pulsar in SN1987A will ultimately spin down to a slowly rotating neutron star. The cross-hatched region is bounded by the limiting relation for Roche models, equation (3), for $P_{\text{rot}} = 0.5$ ms and by the well-known constraint $2M/R \leq 8/9$ that must be satisfied by all non-rotating stars with decreasing pressure and real sound speed in general relativity^{11,12}. This allowed region of non-rotating star models is bounded at low R by the radius of a $1.4M_{\odot}$ model with $M/R = 4/9$; no equation of state with a monotonic (that is, radially stable) M - R relation is consistent with models to the left of this line as well as with a maximum mass above $1.4M_{\odot}$, as observations require (J. H. Taylor and J. M. Weisberg, preprint). The singly hatched region in Fig. 1 is the extra phase-space that would be allowed by the conservative bounds on the maximum rotation rate assuming incompressible stars.

Also shown on Fig. 1 are the M - R relations for selected representative nuclear equations of state^{13,14}. As can easily be seen from the figure, one of the currently viable equations of state has stable non-rotating star models in the cross-hatched region, but just barely; only three have such models in the singly hatched region. (None of the equations of state has non-rotating endpoints with $M \approx 1.4M_{\odot}$, which may be favoured on evolutionary grounds¹⁵, in either region.) Now, we have seen that the relativistic Roche model accurately estimates the maximum rotation rates allowed for a variety of equations of state. We therefore conclude that if the remnant in SN1987A is a neutron star with $P_{\text{rot}} = 0.5$ ms which will ultimately spin down to slow rotation without appreciable loss of material, then all previously viable nuclear equations of state may be ruled out. Moreover, the cross-hatched region in Fig. 1 is the 'comfort zone' for all equations of state proposed in the future. Any equations of state that fail to produce stable non-rotating star models that lie within that zone should be regarded with extreme suspicion. Any equations of state that fail to produce stable non-rotating star models that lie within the singly hatched region may be ignored.

The discovery of a 0.5-ms optical pulsar in the remains of SN1987A therefore demands that the equation of state be based on extremely soft nuclear matter. Pion¹⁶ condensates, and possibly quark matter¹⁷, may now resurface as ingredients of any realistic theory of nuclear matter at neutron-star densities.

A neutron star rotating near the break-up point may be unstable to certain non-axisymmetric modes (ref. 18; J. L. Friedman *et al.*, preprint; J. R. Ipser and L. Lindblom, preprint). The exact onset of this instability depends on details of the microphysics and is not accurately known; current thinking is that it is quite close to the break-up point. Our conclusions are based on equilibrium considerations, not stability. Moreover, they do not depend on details of the equation of state. In this sense they are more general than results from numerical simulations, although slightly less stringent.

Of course there are two outstanding loopholes in this pessimistic argument: the neutron star in SN1987A may in fact be vibrating, not rotating (ref. 19; R. C. Duncan, preprint). Moreover, even if it is spinning at $P_{\text{rot}} = 0.5$ ms, the neutron star may only be temporarily supported, by its rapid rotation, against eventual collapse to a black hole (K. Sato and H. Suzuki, preprint; I. Goldman, preprint; J. L. Friedman *et al.*, preprint). □

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Pressure-induced transition of the hydrogen bond in the ferroelectric compounds KH_2PO_4 and KD_2PO_4

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THE prototypical ferroelectric compound KH_2PO_4 contains hydrogen bonds that exhibit interesting properties. The proton lies in one of two equivalent positions between oxygen atoms of two PO_4 tetrahedra. Below the ferroelectric transition temperature T_c there exists ordering of the protons in one of these two minima. A large increase in T_c is observed when the proton is substituted by a deuteron. Here we report results which show that in both KH_2PO_4 and its deuterated form KD_2PO_4 at room temperature (that is, above T_c), the hydrogen-bond length decreases initially with increasing pressure, but then an abrupt and pronounced elongation occurs at a critical pressure P_c , corresponding to 2.7 and 4.2 GPa for KH_2PO_4 and KD_2PO_4 respectively. At P_c , the bond lengths are nearly equal to those predicted if the proton (deuteron) were to be located in a single minimum at the midpoint of the two oxygens. The P-O bond lengths in the PO_4 tetrahedra also begin to decrease above P_c . These observations suggest that there may be a fairly complex interrelation of the various bond lengths involved in the ferroelectric transition, and reveal a hitherto unsuspected property of the hydrogen bond.

In ferroelectric crystals of this type, hydrogen-bond length is thought to be closely related to the equilibrium proton positions. It has been predicted that, as the oxygen-oxygen distance between adjacent PO_4 tetrahedra, $R_{\text{O-O}}$, decreases, so does the separation of the two potential minima for the proton, until finally the proton is located at a single minimum at the midpoint of the bond, without realization of a phase transition.

The T_c of KH_2PO_4 (KDP) is 122 K, whereas that of KD_2PO_4 (DKDP) is 229 K at atmospheric pressure¹. Samara^{2,3} has demonstrated that both KDP and DKDP show a large negative

pressure dependence of T_c , and the T_c of KDP falls to 0 K at 1.7 GPa. In single-crystal neutron diffraction experiments on KDP and other isomorphous substances under pressure^{1,4-8} results for KDP indicated that R_{O-O} at 1.65 GPa and room temperature was lower than its value at ambient pressure and temperature; it was suggested that a proton could not be localized at only one site because of the zero point motion at 1.7 GPa and 0 K—that is, the ferroelectricity diminishes above 1.7 GPa. On the other hand, Ichikawa *et al.*⁹ empirically showed that for some KDP-type crystals with tetragonal symmetry, T_c depends linearly on the hydrogen-bond length, irrespective of pressure.

In this study, a modified Merrill-Bassett type of diamond cell¹⁰ mounted on a four-circle diffractometer was used for single-crystal X-ray structure analysis under high pressure. A fluid mixture of methanol and ethanol (4:1 v/v) was used to generate hydrostatic pressure, which was determined by a ruby fluorescent method. The single-crystal sample had dimensions of $\sim 150 \times 150 \times 70 \mu\text{m}$ parallel to the a , c and b axes, respectively. The ac plane was glued onto the culet face of one of the diamond anvils with a small amount of silicone grease. Mo $K\alpha$ radiation from a rotating-anode high-power X-ray generator was made monochromatic with pyrolytic graphite. Before the high-pressure experiments, diffraction data were obtained for a crystal at atmospheric pressure (0.0001 GPa) both with and without the diamond cell; we attributed differences between the intensities of reflections with the same index to X-ray absorption by the diamond anvils and beryllium disks of the cell. The intensity of reflections measured at high pressure was corrected for absorption by taking account of the difference between the two data sets at atmospheric pressure, because the X-ray path through the cell was almost the same for reflections with the same index at individual pressures.

Lattice parameters at high pressures were determined using 2θ values for 10–14 reflections in the range 40° – 50° . The intensity measurements obtained by the θ – 2θ scan technique were made with a scan speed of 3° per min in 2θ for reflections in a quadrant sphere of reciprocal space within $2\theta \leq 60^\circ$. Independent reflections at each pressure were obtained by averaging equivalent reflections.

The possibility of a symmetry change from space group $I\bar{4}2d$ for KDP at P_c was investigated using two X-ray diffraction methods: oscillation photography and powder diffraction for 2θ angles between 5° and 35° , measured in an angle-dispersive system using a combination of Mo $K\alpha$ radiation with a solid-state detector. No pattern change suggestive of a symmetry different from that at low pressure was observed at pressures

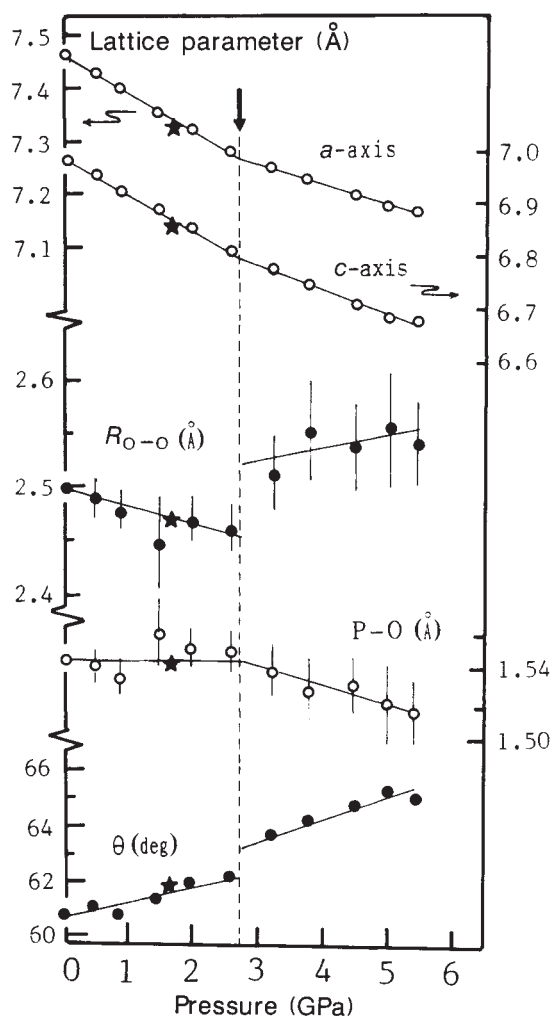


FIG. 1 Crystallographic data of KH_2PO_4 as a function of pressure at room temperature: lattice parameters; hydrogen-bond length, R_{O-O} ; P—O distance in the PO_4 tetrahedron and its rotation angle, θ , around the 4-fold axis. Arrow and dotted line indicate pressure at which the different structural changes can be observed. Values at 1.65 GPa from Tibbals *et al.*⁶ are plotted (asterisks) for comparison.

TABLE 1 Crystallographic data for KDP under different pressures at room temperature

P (GPa)	0.0001	0.4	0.9	1.4	2.0	2.6	3.2	3.8	4.5	5.0	5.4
Lattice parameter											
a (Å)	7.4596 (11)	7.425 (2)	7.395 (2)	7.351 (2)	7.319 (2)	7.278 (2)	7.247 (1)	7.225 (4)	7.197 (1)	7.174 (5)	7.163 (2)
c (Å)	6.9795 (9)	6.957 (2)	6.921 (2)	6.886 (2)	6.849 (2)	6.807 (3)	6.774 (1)	6.747 (3)	6.706 (1)	6.681 (5)	6.676 (2)
Positional parameter											
x_O	0.1478 (3)	0.1496 (12)	0.1483 (11)	0.1535 (24)	0.1531 (13)	0.1539 (15)	0.1552 (20)	0.1556 (25)	0.1568 (22)	0.1566 (30)	0.1564 (22)
y_O	0.0826 (3)	0.0825 (14)	0.0828 (13)	0.0839 (29)	0.0815 (15)	0.0812 (17)	0.0769 (25)	0.0736 (32)	0.0739 (29)	0.0721 (40)	0.0728 (28)
z_O	0.1260 (4)	0.1244 (12)	0.1257 (11)	0.1273 (22)	0.1287 (14)	0.1297 (15)	0.1296 (21)	0.1298 (25)	0.1307 (23)	0.1312 (29)	0.1301 (23)
Temperature factor											
B_K	1.58 (2)	1.8 (2)	1.7 (2)	1.6 (4)	1.4 (2)	1.4 (3)	1.8 (4)	1.1 (4)	1.4 (4)	1.4 (5)	1.3 (4)
B_P	1.14 (2)	1.3 (2)	1.4 (2)	1.2 (4)	1.3 (2)	1.2 (3)	1.6 (4)	1.0 (4)	1.0 (4)	0.6 (5)	1.1 (4)
B_O	1.39 (3)	1.8 (3)	2.0 (2)	1.8 (6)	1.8 (3)	1.7 (3)	2.5 (5)	1.7 (6)	2.0 (5)	1.7 (8)	2.1 (5)
Number of reflections	300	35	35	34	34	35	35	27	26	24	28
R (%)	5.7	3.4	3.6	6.5	4.2	4.7	5.7	5.1	4.5	5.0	4.7
wR (%)	5.8	3.9	3.8	7.4	4.6	4.9	6.7	6.3	5.3	6.7	5.9
R_{O-O} (Å)	2.498 (4)	2.488 (21)	2.473 (19)	2.443 (43)	2.467 (22)	2.457 (24)	2.509 (36)	2.550 (47)	2.536 (42)	2.554 (57)	2.539 (41)
P—O (Å)	1.539 (2)	1.535 (91)	1.528 (81)	1.556 (18)	1.545 (99)	1.544 (11)	1.532 (15)	1.521 (18)	1.525 (16)	1.516 (22)	1.510 (16)
O—O in PO_4 (Å)	2.526 (4)	2.536 (19)	2.512 (17)	2.571 (37)	2.539 (20)	2.533 (22)	2.511 (31)	2.487 (38)	2.495 (33)	2.473 (46)	2.472 (34)
O—P—O (deg)	110.3 (2)	111.4 (7)	110.6 (6)	111.4 (12)	110.4 (7)	110.3 (8)	110.1 (11)	109.7 (13)	109.8 (12)	109.3 (16)	109.8 (12)
θ (deg)	60.80	61.12	60.82	61.35	61.97	62.18	63.64	64.69	64.75	65.27	65.03
ϕ (deg)	0.3074	0.2051	0.2261	0.7397	1.174	1.479	1.417	1.446	1.725	1.859	1.525

The rotation angle of a PO_4 tetrahedron around the 4-fold axis is denoted by θ and ϕ is the inclination of the hydrogen bond to (001). Space group is $I\bar{4}2d$ and the positional parameters are $P(0, 0, 0)$, $K(0, 0, 1/2)$ and $O(x_O, y_O, z_O)$. Numbers in parentheses denote standard deviations.

TABLE 2 Crystallographic data for DKDP under different pressures at room temperature

P (GPa)	0.0001	1.3	2.7	3.3	3.9	4.5	4.9	5.2	5.6	6.5
Lattice parameter										
a (Å)	7.4696 (4)	7.375 (5)	7.297 (9)	7.264 (3)	7.226 (7)	7.201 (6)	7.186 (1)	7.199 (3)	7.170 (1)	7.155 (2)
c (Å)	6.9750 (4)	6.874 (3)	6.974 (2)	6.762 (2)	6.713 (5)	6.698 (4)	6.680 (1)	6.673 (3)	6.667 (1)	6.641 (5)
Positional parameter										
x _O	0.1489 (2)	0.1502 (23)	0.1581 (29)	0.1556 (33)	0.1576 (66)	0.1574 (63)	0.1530 (19)	0.1630 (70)	0.1577 (27)	0.1516 (30)
y _O	0.0809 (2)	0.0795 (20)	0.0818 (30)	0.0783 (36)	0.0819 (67)	0.0749 (77)	0.0731 (21)	0.0713 (91)	0.0705 (31)	0.0697 (36)
z _O	0.1260 (2)	0.1305 (17)	0.1289 (24)	0.1288 (29)	0.1348 (65)	0.1243 (53)	0.1316 (19)	0.1315 (91)	0.1272 (30)	0.1361 (31)
Temperature factor										
B _K	1.61 (2)	1.1 (2)	2.3 (2)	1.8 (2)	1.8 (2)	1.2 (3)	1.6 (3)	1.7 (3)	1.2 (4)	0.8 (4)
B _P	1.20 (2)	1.8 (2)	1.2 (2)	2.4 (3)	1.7 (2)	2.4 (4)	1.2 (3)	1.5 (4)	1.8 (5)	2.5 (5)
B _O	1.40 (3)	2.5 (2)	2.6 (2)	2.6 (3)	2.5 (3)	2.6 (3)	1.8 (4)	2.3 (6)	2.5 (5)	1.0 (5)
Number of reflections										
R (%)	5.1	4.9	7.3	6.5	7.0	7.2	7.3	7.3	6.9	7.5
wR (%)	4.4	5.2	7.0	6.4	6.1	6.9	5.9	6.8	4.9	5.3
R _{O-O} (Å)	2.5263 (30)	2.510 (29)	2.449 (47)	2.475 (52)	2.433 (70)	2.540 (70)	2.554 (30)	2.582 (80)	2.574 (44)	2.584 (52)
P-O (Å)	1.5410 (15)	1.544 (15)	1.554 (20)	1.533 (22)	1.557 (26)	1.516 (44)	1.509 (13)	1.482 (42)	1.501 (20)	1.497 (22)
O-O in PO ₄ (Å)	2.5316 (30)	2.522 (23)	2.597 (42)	2.531 (49)	2.567 (96)	2.510 (95)	2.437 (28)	2.562 (91)	2.477 (40)	2.388 (45)
O-P-O (deg)	110.5 (1)	109.0 (10)	109.0 (1)	110.8 (16)	109.0 (10)	112.7 (30)	108.3 (10)	108.6 (8)	111.2 (15)	108.3 (17)
θ (deg)	61.48	62.12	62.65	63.18	62.55	64.55	64.47	65.36	65.90	65.32
φ (deg)	0.313	0.868	1.232	1.193	1.548	1.982	2.004	2.118	2.656	3.256

The deuteration for the DKDP sample used here is 99.7%. Symbols as in Table 1. Numbers in parentheses denote standard deviations.

above 2.7 GPa. Therefore, we refined the structural parameters using the least-squares program LINUS on the basis that the KDP and DKDP space groups are $I42d$ at all pressures. We adopted the parameters determined at atmospheric pressure by Nelmes *et al.*¹ as our initial values. Atomic scattering factors for neutral atoms were taken from ref. 11. Results for KDP and DKDP are summarized in Tables 1 and 2 respectively.

At ~2.7 GPa, a bending in the lattice parameter of KDP is observed, which must reflect a structural change in the crystal (Fig. 1). One remarkable change is seen in the length of the hydrogen bond, R_{O-O} , that connects two adjacent PO₄ tetrahedra. The bond length decreases almost linearly with increasing pressure, but at pressures greater than 2.7 GPa it becomes much longer. On the other hand, the P-O distance in the PO₄ tetrahedron is kept almost constant up to 2.7 GPa, above which it begins to shrink. We recognized no significant pressure dependence in the O—P—O angle of the PO₄ tetrahedron within the accuracy of our refinement. The rotation angle of the PO₄ tetrahedron around the 4-fold axis parallel to the *c* axis, represented as θ , increases over the whole pressure range. There may be a small but abrupt increase in θ at 2.7 GPa. The small inclination angle ϕ of the hydrogen bond to (001) is also listed in Table 1; it is seen to increase with pressure. In Fig. 1 we show for comparison the values obtained by Tibballs *et al.*⁶ at 1.65 GPa and room temperature using a single-crystal neutron-diffraction technique; these are in good agreement with our results. Under pressure, the lattice parameters, interatomic distances and angles of DKDP behave as in KDP, although there are some differences at 4.2 GPa for DKDP.

The results for KDP and DKDP are illustrated schematically in Fig. 2. The rotation of the PO₄ tetrahedron at pressures up to P_c results in a decrease in R_{O-O} which is smaller than would be expected from the decrease in the *a*-axis, as already noted by Meyer *et al.*⁴. On the other hand, the elongation of R_{O-O} , which is almost parallel to (001) at pressures above P_c is induced against the progressive decrease of the *a*-axis, and is associated with a decrease in P—O distance and an increase in θ . This remarkable elongation of the hydrogen bond at pressures above P_c is unexpected. Matsushita and Matsubara¹² calculated the relation between the O—H(D)···O and O—H(D) distances by making use of a simple model potential, in which two Morse functions are placed back-to-back to represent the interatomic potential arising from the action on a hydrogen atom of two neighbouring oxygen atoms. Moreover, they introduced a tunnelling motion of the H atom as a quantum effect in their calculation. Their results show that, for a sufficiently large R_{O-O} , the H atom moves in a potential with two minima, but that at

a certain critical distance the potential becomes a single minimum. The R_{O-O} distance at which the two minima converge to a single minimum was calculated to be 2.47 Å for O—H···O and 2.44 Å for O—D···O. In Fig. 1, which shows the behaviour of R_{O-O} for KDP under pressure, solid lines are drawn by the least-squares method for two pressure regions above and below P_c . The line in the lower pressure region gives a value of 2.45 Å for R_{O-O} at 2.7 GPa. Similarly, a value of 2.44 Å is obtained at 4.2 GPa for DKDP. These distances are close to those calculated by Matsushita and Matsubara for the realization of a single-minimum potential.

Coulson¹³ has suggested that when the O···O distance is as low as ~2.4 Å, the proton resides at the centre of the bond, which then becomes very different in character from a normal O—H bond. Indeed, the bond does become very different when the proton or deuteron is at the centre in KDP or DKDP; the sudden elongation of R_{O-O} at P_c follows. An unexpected and probably very important feature of the hydrogen bond has been

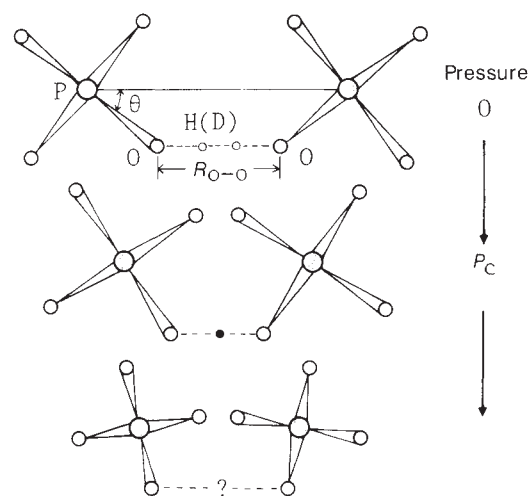


FIG. 2 Schematic diagram showing the structural change of KH₂PO₄ and KD₂PO₄ under pressure, projected to (001). Only part of the structure is shown. Initially, with increasing pressure, the hydrogen bond decreases in length and the PO₄ tetrahedron gradually rotates around the 4-fold axis. But at pressures above P_c , when H(D) is assumed to be located at the midpoint of the hydrogen bond the bond becomes greatly elongated and there is shrinking of the PO₄ tetrahedron, the rotation of which still continues.

revealed. The concept of hydrogen bonding being mediated by a hydrogen atom between ions can be used to explain its unexpected behaviour under extreme conditions such as high pressure.

Our results show that the origin of ferroelectricity, by which the large isotope effect on T_c is explained, should be considered in terms of a combined change in the rotation angle of the PO_4 tetrahedron, the P—O distance and the $R_{\text{O—O}}$ distance, all of which are strongly interrelated. The nature of the dramatic structural change of KDP and DKDP at pressures above P_c needs further investigation; dielectric constant measurements and neutron diffraction studies under pressure should eventually give us more information about this event. $\square$

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Non-parallel chains in crystalline γ -isotactic polypropylene

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CRYSTALLINE synthetic polymers generally exhibit highly anisotropic arrangements of the polymer chains, which tend to lie parallel to each other. Indeed, chain-axis parallelism has become virtually an unchallenged principle of polymer crystallization, essentially for two reasons. First, it seems consistent with the requirements of the close packing of rods, which may be assumed to approximate crystallized polymer chains, and second, most polymer crystal structures have been determined from samples that are fibrous. As part of a study^{1–4} of crystal structures of polymer phases that do not readily form fibrous specimens, we have determined the crystal structure of the γ -form of isotactic polypropylene (γ -iPP)^{5–8}. We show that the structure comprises layers which are two chains wide, reminiscent of the α -phase⁹, but with the chain-axis directions in adjacent bilayers at an angle of 80° to one another. This feature recalls the arrangement, at the same inter-helical angle, that is proposed to occur at the branching points in α -iPP crystals^{10–12}. Similar packing at large interaxial angles between isochiral chain fragments is also often found in globular proteins¹³. The γ -iPP crystal structure is unusual, however, in that non-parallel chains appear in a fully ordered array. The driving force for such packing seems to be the favourable registration between helices that occurs for this tilt angle.

Isotactic polypropylene, synthesized using the homogeneous catalyst methylalumoxane-rac-ethylenebis(4,5,6,7-tetrahydro-1-indenyl) dichlorozirconium¹⁴, was supplied by L. Resconi. Details of the preparation and of the gel-permeation chromatography (GPC) and NMR characterization are fully

described in ref. 15, sample E. The mean molecular mass was 6,300 with a polydispersity of 1.8 and an acceptable degree of isotacticity, as determined by NMR. The dried polymer, as polymerized, displayed α -phase crystallinity. After melting the sample in an evacuated glass vial, it was cooled very slowly to 115°C and kept at this temperature for 48 h, before being slowly cooled to room temperature.

The X-ray diffraction spectra (Fig. 1) of the recrystallized iPP sample show a high degree of γ -crystallinity; no traces of other crystalline phases are apparent. The diffraction maxima are sharper than in previously published spectra of γ -iPP and the unit-cell parameters have therefore been more accurately determined. The previously reported triclinic lattice¹⁶ ($a = 6.54 \text{ \AA}$, $b = 21.40 \text{ \AA}$, $c = 6.50 \text{ \AA}$, $\alpha = 89^\circ$, $\beta = 99.6^\circ$, $\gamma = 99^\circ$; cell I), is in

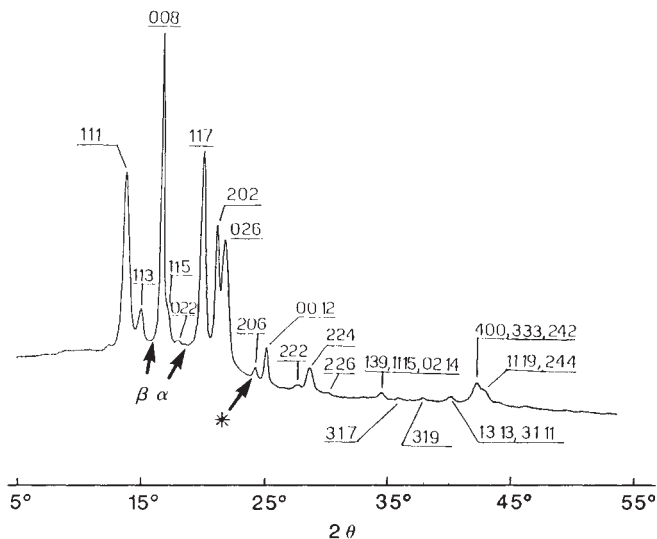


FIG. 1 The experimental X-ray diffraction profile of γ -iPP. Expected locations of major α - and β -phase diffraction maxima are marked by arrows; the peak marked with * cannot be indexed by cell I. Indexing with cell III is also shown. X-ray diffraction profiles were recorded at room temperature with a Siemens D500 diffractometer equipped with a step-scan attachment and Soller slits, using Ni-filtered $\text{Cu K}\alpha$ radiation. The step width was $0.30^\circ (2\theta)$ and the 2θ range was $4\text{--}50^\circ$. The details of the data recording and handling are given in refs 1–4.

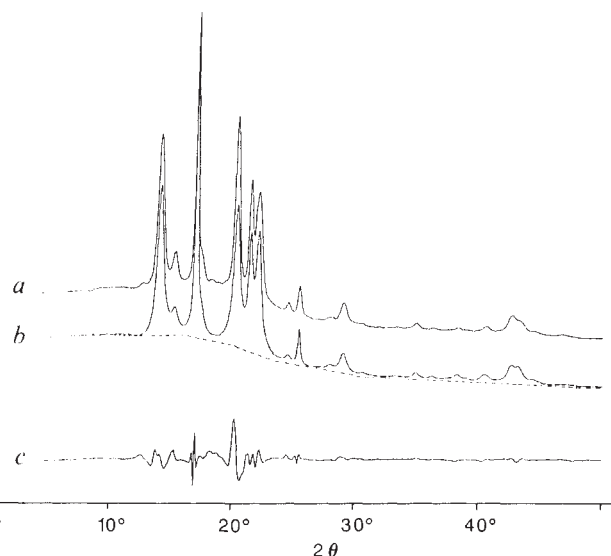


FIG. 2 a, The observed powder X-ray diffraction profile of γ -iPP compared with b, the calculated profile. Curve c represents the difference profile, and the dashed line is the background contribution.

satisfactory agreement with the $hk0$ (equatorial) and the $h0l$ electron diffraction data¹⁷, the main features of the X-ray diffraction spectra and the observed density. Cell I fails to account, however, for the X-ray diffraction peak observed at $2\theta = 24.35^\circ$ (Fig. 1). Furthermore the 21.1° peak is indexed as (101) and (111), but published electron diffraction patterns, (ref. 17, Fig. 5), show it to consist of two equal-intensity reflections differing by two, not one, k units. By imposing the latter requirement and indexing the reflection at $2\theta = 24.35^\circ$ as (121) (the calculated value for cell I is 23.7°), the following reduced triclinic unit cell was derived: $a = 6.55 \text{ \AA}$, $b = 21.57 \text{ \AA}$, $c = 6.55 \text{ \AA}$, $\alpha = 97.4^\circ$, $\beta = 98.8^\circ$, $\gamma = 97.4^\circ$ (cell II). The density calculated for this cell, 0.93 g cm^{-3} , is in satisfactory agreement with the measured value. Extensive attempts were made to determine and refine models maintaining chain parallelism in the triclinic unit cell II. A number of packing arrangements were tested, both in space group $P\bar{1}$, with two independent chains, and in $P1$, with four independent chains. Statistical anticlinical substitution, that is, random coexistence of chains with opposite ('up' or 'down') orientation of the side groups, was taken into account but results were always unacceptable because the 2θ region $18\text{--}24^\circ$ could never be adequately reproduced.

The crystallographic data are consistent with a face-centred orthorhombic lattice having $a = 8.54 \text{ \AA}$, $b = 9.93 \text{ \AA}$, $c = 42.41 \text{ \AA}$ (cell III). In this unit cell the chains lie parallel to both the diagonals in the a - b plane (which measure $13.08 \text{ \AA}$, that is, twice the iPP helical periodicity) and form an angle of 81.2° , a value which is close to the one formed by the chain axes in branching α -iPP crystals. We also note that published^{16,17} γ -iPP electron diffraction patterns display full orthorhombic symmetry.

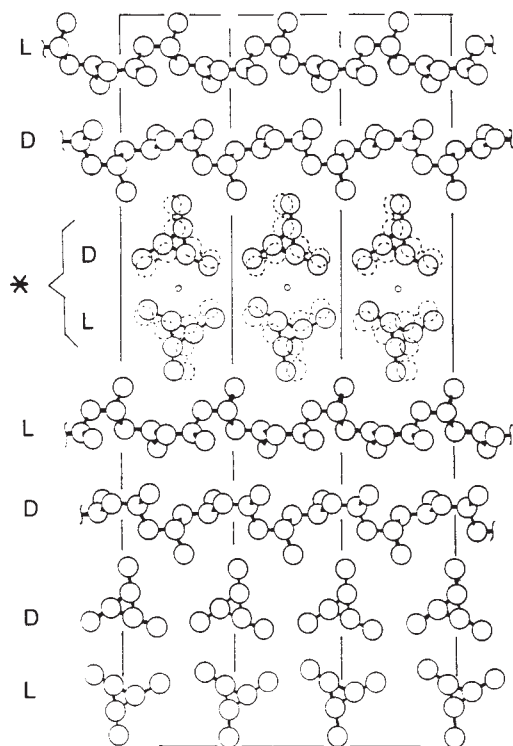


FIG. 3 Packing of γ -iPP, viewed in cell III along one diagonal of the a - b plane. For the sake of clarity, coaxial anticlinical helices are shown (dashed) only for some chains. The figure shows the basic features of mutual chain arrangement and methyl-group interdigitation. The labels D and L refer to the chirality of the helices belonging to the corresponding layer, and an asterisk identifies a bilayer of parallel chains.

Space groups $Fddd$ and $Fdd2$ have appropriate symmetry elements for cell III, but we focused on $Fddd$ because it gives the best agreement with experimental data. Acceptable packing and the qualitative features of the X-ray diffraction profile impose severe restrictions on possible models; as well as being parallel to the a - b plane diagonals, the chain axes must intersect an orthogonal twofold axis, and they have to be stacked in layers separated by approximately $c/8 = 5.3 \text{ \AA}$. The statistical coexistence of anticlinical chains satisfies these requirements. To match the experimental density, 32 axial 3_1 helical repeats with an occupancy factor of 0.5 have to be placed in the unit cell, and only one independent threefold helical unit is required to describe the whole structure. Here, refinement according to the constrained Rietveld method¹⁸ amounts almost to a rigid-body treatment of the iPP threefold helix. The agreement between calculated and observed profiles (Fig. 2), is good. Atomic coordinates of the independent monomer unit are given in Table 1. An assessment of the reliability of the refined model can be obtained by comparing the value of the net intensity disagreement factor¹⁸ in the present case ($R_2^2 = 0.152$), with the best one obtained after similar refining of the widely accepted structure of α -iPP¹⁸ ($R_2^2 = 0.154$). The γ -iPP crystal structure is shown in Fig. 3; the projection is along one of the a - b plane diagonals, which allows helices with different orientations to be clearly distinguished (coaxial anticlinical molecules are omitted for clarity). Although the structural model was hitherto refined only against the X-ray diffraction profile, no overly close contacts are observed.

The overall architecture of the γ -iPP crystal structure, although presenting the striking novelty of non-parallel chains has similarities to the α -phase. The independent unit in both

TABLE 1 Crystallographic data

a, Refined fractional atomic coordinates for carbon atoms of one monomer unit. The complex helix is generated by a threefold screw axis.

	x/a	y/b	z/c
CH ₂	-0.1489	-0.0934	0.0441
CH	-0.1139	-0.0327	0.0767
CH ₃	-0.2665	0.0137	0.0924

b, Comparison of observed and calculated integrated intensities for γ -iPP. Observed values have been estimated with reference to the calculated base-line (dashed line in Fig. 2).

hkl	2θ ($^\circ$)	d ($\text{\AA}$)	I_o	I_c
0 0 4	8.34	10.60	0	1
1 1 1	13.84	6.40	100	98
1 1 3	15.05	5.89	15	14
0 0 8	16.72	5.30	74	78
1 1 5	17.23	5.15	11	12
0 2 2	18.35	4.83	0	0
1 1 7	20.07	4.42	85	74
2 0 2	21.22	4.19	40	38
0 2 6	21.88	4.06	63	66
1 1 9	23.35	3.81	0	0
2 0 6	24.35	3.66	3	5
0 0 12	25.20	3.53	9	11
1 1 11	26.95	3.31	1	1
2 2 0	27.55	3.23	1	1
0 2 10	27.66			
2 2 2	27.88	3.20	2	1
2 2 4	28.83	3.10	9	12
1 3 1	29.01	3.08	2	3
1 3 3	29.63	3.01	0	0
2 0 10	29.69			
2 2 6	30.37	2.94	2	1
3 1 3	33.35	2.69	1	1
1 1 15	34.62	2.58	3	4
0 2 14	34.69			
1 3 9	34.75			

structures is a single 6.5-Å axial repeat, with bilayers of molecules—related by glide symmetry and thus of opposite chirality—being found in both phases. Indeed it seems reasonable to suggest that bilayers (Fig. 3) in γ -iPP are a consequence of the coexistence of helices of opposite chirality. Again, 'up-down' statistical disorder is an essential feature of both the α - and γ -structures. Furthermore, the 80° tilt between adjacent layers of isochiral helices in γ -iPP cannot be considered to be totally unexpected—similar arrangements have been postulated^{10,11} to explain, at a molecular level, the tilt angle of ~80° observed in α -iPP branching crystals. In the latter, tilting occurs only at (disordered) branching sites, whereas it is a feature of the regular crystallographic structure in γ -iPP. It breaks the sequence of alternating layers of all left- and right-handed helices, placing isochiral helices in contiguous layers. An analogy may be drawn with helix-to-helix packing in globular proteins, where an interaxial angle of ~50° is most often observed¹³, indicating that favourable ridge-to-groove juxtaposition of isochiral helices is in general expected to induce a strong angular deviation in the interacting chain axes. In globular proteins this feature is local but in γ -iPP the tilt angle of 80° involves entire crystalline bilayers. The reason for this may be the near equality of the helix repeat length and the distance between adjacent parallel isochiral chains in a layer (~6.5 Å).

The driving force leading to the γ -iPP structure is not clear. It is unlikely to be kinetic because of the complex architecture of this phase, the fact that γ -iPP is obtained by slow cooling

and at high pressure, and the reduced importance of chain folding^{17,19}. Thus crystallization of γ -iPP appears to be related to its stability¹⁹, that is, to the favourable packing energy that results from registry of the inclined chains; the melting of the γ -phase at a lower temperature than the α -phase could simply be due to smaller crystallite size¹⁷. □

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Biotic enhancement of weathering and the habitability of Earth

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AN important question in the Earth sciences is the role of the biota in the chemical weathering of silicate rocks, which affects atmospheric CO₂ and therefore climate^{1–10}. No comprehensive study of biotic influences, however, has quantitatively examined the climatic consequences were weathering to take place under completely abiotic conditions. Here we calculate that if today's weathering is 10, 100 or 1,000 times the abiotic weathering rate, then an abiotic Earth would be, respectively, ~15, 30 or 45 °C warmer than today. The upper two temperatures are preferred estimates because of the probable almost complete absence of soil under abiotic conditions, suggesting that without a biota that significantly enhances weathering rates, the Earth today would be uninhabitable for nearly all but the most primitive microbes. Life may have been crucial in cooling early Earth and maintaining relatively cool conditions.

As well as having a role in elevating the partial pressure of CO₂, p_{CO_2} , in the soil⁸, the biota (from microbes to vascular plants) accelerates chemical weathering by stabilizing soil, a reservoir of high surface area, which acts as a sponge for water and thus as a medium for acid attack^{10–13}. Other important roles of biota in accelerating weathering include producing humic and other organic acids, inorganic acids and chelating agents, acting as a sink for the nutrients freed by weathering, and contributing to physical weathering through microfracturing of mineral grains¹⁴.

Polynov¹⁵, as long ago as 1953, emphasized the importance of biotic amplification of weathering. Indeed, organic acids may be essential to give the observed weathering rates because of the slow kinetics of proton production in the CO₂–H₂O system¹⁶.

Whereas the emergence of vascular plants in the Silurian almost certainly accelerated chemical weathering¹⁰, microbial and possibly lichen colonization of the continents in the Precambrian^{12,17} probably resulted in a considerable increase in chemical weathering relative to abiotic conditions at the same atmospheric p_{CO_2} .

The chemical dissolution rates increase with p_{CO_2} as $(p_{\text{CO}_2}/p_{\text{CO}_2}^0)^\alpha$ where $p_{\text{CO}_2}^0$ is the present p_{CO_2} in the atmosphere and α is between 0.3 and 0.4. A value for α of 0.3 used in previous studies^{2,18,19} was derived from experiments on alkali feldspars. From H₂O–CO₂ equilibria, a_{H^+} varies with $(p_{\text{CO}_2})^{0.5}$, assuming continuous reaction of the minerals with fresh water saturated with CO₂. The rate of dissolution of silicates varies with $(a_{\text{H}^+})^n$ where $0 \leq n \leq 1$, and n varies from 0.54 (anorthite) to 1.0 (forsterite) for Ca, Mg silicates¹⁶ corresponding to an α between 0.27 and 0.5, with 0.38 being the mean value for anorthite, forsterite, enstatite and diopside. We suggest that α , for Ca, Mg silicates exposed on the continents of an abiotic Earth, is probably between 0.3 and 0.4. Root respiration and soil biota generate a soil p_{CO_2} that is 10–100 times the level in the atmosphere⁸. Thus, if the p_{CO_2} elevation in soil was the only factor in chemical weathering enhancement, weathering rates in soils should be only 10^{0.3}–100^{0.4} or 2–6 times the rate on bare rock under similar temperature and rainfall conditions; an elevation of this order, by itself, would not have a major effect on planetary habitability²⁰. We will demonstrate, however, that an appreciable additional enhancement in the weathering apparently occurs due to the other biotic factors mentioned above, including of course the soil effect.

Sterile conditions no longer exist naturally on the surface of the Earth so estimates of the rates of abiotic weathering must be made indirectly. Although today's rocks, or rocks dating back as far as the Precambrian, are not models for weathering under abiotic conditions, data from field studies and experiments can be used to set limits on biotic enhancement of weathering. Let us first consider weathering on a two-dimensional surface without the amplification as a result of the small particle size/large surface areas in a soil. Laboratory experiments on Ca, Mg silicates give a maximum dissolution rate of $\leq 5 \times 10^{-5}$ mm yr⁻¹ at 25 °C under the conditions of continuous

contact with undersaturated water, calculated for pH 5.66 (equilibrium with present p_{CO_2})¹⁶. This rate is almost certainly a maximum for natural abiotic weathering of a bare rock surface at the present p_{CO_2} for the following reasons.

First, bare rock in nature, indeed mineral grains in soil, are not in continuous contact with water. This effect reduces the dissolution rate by the fraction of time that there is water contact¹⁶. Second, laboratory preparation of samples, particularly grinding, raises dissolution rates by creating surface deformation and dislocations²¹. Indeed, the natural dissolution rate of silicates without enhancement by organic chelation may be one to three orders of magnitude lower than the rate quoted above²², possibly because bulk dissolution rates may vary little with grain size because of the decrease in the ratio of defect sites to surface area with progressive attack²³.

A study of Hawaiian basalt flows of known age²⁴ indicates that there is an acceleration of chemical weathering on the order of at least 10 to 100 times for lichen covered surfaces relative to bare rock. The chemical weathering enhancement in tropical soils is four to ten times that due to lichen colonization of the Hawaiian lavas, similar to the enhancement found in Iceland,⁹ in a comparison of the bicarbonate levels in rivers traversing 'barren' (that is, lichen colonized land¹¹) and areas of vegetation. For the oldest flow (60-years old at time of collection) under conditions of moderate to heavy rainfall (127–191 cm yr⁻¹) the rate of rind development was $<3 \times 10^{-5}$ mm yr⁻¹ on bare rock. An even lower weathering rate (average of $<5 \times 10^{-6}$ mm yr⁻¹) is derived from a study of rind development on andesitic and basaltic clasts from the western U.S.A. in the last 500,000 years²⁵.

We conclude that the chemical weathering rate of a two-dimensional rock surface of the continental crust under abiotic conditions with similar temperature and rainfall conditions as the Hawaiian case is $\leq 5 \times 10^{-5}$ mm yr⁻¹, perhaps even one to three orders of magnitude lower. Of course, weathering in a soil

environment dramatically increases the chemical denudation rate relative to bare rock as a result of much higher surface areas of exposed minerals for a given land area. The typical chemical denudation rate in tropical and saprolitic soils is 0.04–0.05 mm yr⁻¹ (refs 26, 27) or $\geq 10^3$ times the inferred abiotic bare-rock rate. An amplification of this order can probably be accounted for by soil formation with p_{CO_2} elevation and other biotic influences.

But would soils be formed and stabilized under abiotic conditions? Frost wedging, a particularly effective mechanism of mechanical weathering would be unlikely at the high temperatures prevailing on an abiotic Earth (see later calculations). Other mechanisms of mechanical weathering, such as cracking from clay and salt formation²⁸, may well be amplified at present by microbial biota retaining water in cracks, although joint formation and cracking from thermal stresses would still occur under abiotic conditions. We suspect that the amplification of weathering from the latter effects is either small compared to that resulting from the presence of soil itself, or that the two-dimensional abiotic rock surface rate is indeed one to three orders of magnitude lower than 5×10^{-5} mm yr⁻¹—otherwise tropical and saprolitic soil chemical denudation rates would be several orders of magnitude higher, from the combined amplifications, than those observed (the soil effect itself must contribute several orders of magnitude of amplification).

We propose that soil formation on Earth would probably be largely prevented by the rapid erosion due to running water and wind in the absence of a stabilizing crust of algae, lichens or more advanced biota (see refs 11–13 for discussion of this role of biota), as well as the absence of biotic contribution to physical weathering. Soils would probably not form at all in mountainous regions, precisely those sites where the most rapid chemical denudation now occurs because of a combination of mass movement exposing fresh rock and vegetation-anchoring soil²⁹. Some

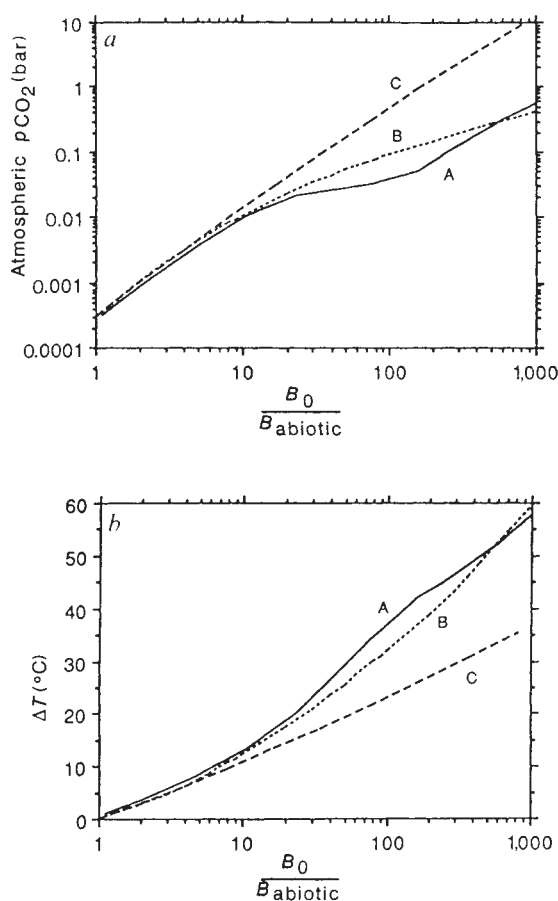


FIG. 1 *a*, Atmospheric CO_2 (p_{CO_2}) and *b*, average global surface temperature (T , expressed as deviation (ΔT) from today's T) as a function of B_0/B_{abiotic} (see equation (1)) the enhancement of present biotic weathering relative to an abiotic weathering rate, under the same conditions of p_{CO_2} and T . The plots indicate what today's Earth might have been like without the colonization of the continents by biota. Results were calculated from equation (1) with the following conditions: $V/V_0=1$ and $A/A_0=1$ (present Earth conditions), $\alpha=0.4$ to minimize the ΔT and p_{CO_2} values for the abiotic world (also see text), $\beta=0.056$ from ref. 2, and $\gamma=0.017$ from ref. 2. Estimates of β range from 0.046 (ref. 19) to 0.056 (ref. 2). The lower value is inferred from the observed temperature dependence of K^+ or silica in river water, while the higher is from laboratory experiments on albite. We believe the higher estimate of β is preferable for an abiotic model as it corresponds, for a temperature range of 100 °C, to an activation energy of ~ 50 kJ/mole, a reasonable estimate for a mean activation energy for Ca, Mg silicates of the continental crust (which ranges from 35 kJ mol⁻¹ for anorthite to 79 kJ mol⁻¹ for augite; see ref. 16). Using the higher estimate of β results in a lower computed ΔT for an abiotic model. Higher values for γ have been used¹⁸, but since we set $e^{\gamma(\Delta T)} \leq 2$ because of energy constraints¹⁸, the results for highest values of B_0/B_{abiotic} do not depend on γ . Curves A, B, and C, use the greenhouse relations for T as a function of p_{CO_2} from, respectively, refs 32, 2, and 19. Kasting and Ackerman³² presented results for $T(p_{\text{CO}_2})$ using a full radiative-convective model with p_{CO_2} as high as 100 bar. Walker, Hays, and Kasting² derived a greenhouse equation by parameterizing effects of CO_2 and water vapour, and although this cannot be preferred to results from a radiative-convective model, we present it here because, interestingly, it gives results so close to the model of ref. 32. The third greenhouse model from Marshall *et al.*¹⁹ is from a parameterization of radiative-convective model results from Kuhn up to 0.1 bar CO_2 . Results in the plots above 0.1 bar for C should be treated, therefore, with caution and we present them only as an alternative formulation and a possible lower bound to the calculation. Significant differences in the details of the treatment of water vapour exist in the radiative-convective models (curves 'A' and 'C'). To date, the model from ref. 32 is apparently the only one to calculate temperature with the high values of p_{CO_2} shown. The behaviour of atmospheric water vapour is crucial and is an important unknown (J. Kasting, personal communication; W. Kuhn, personal communication), but we believe that our results represent the best available calculations.

soils might form in lowlands, but the complete absence of any vegetative cover would allow easy erosion even on small slopes. Even today, changes of land use from forest to cultivation result in an orders-of-magnitude increase in sediment yield³⁰. Moreover, because of the lack of water storage under abiotic conditions, compared to that of areas of vegetation, the lowlands could be virtual deserts with most rainfall concentrated in mountain ranges. The enhancement factor of today's Earth relative to that of an abiotic Earth with some abiotically-stabilized soil would be less than the enhancement of $\geq 10^3$ relative to an abiotic Earth of bare rock. The degree of soil stabilization in an abiotic Earth is of course uncertain; we believe that the available evidence supports a low estimate. If under abiotic conditions, say $\leq 10\%$ of the land area was covered by soil with a weathering enhancement factor of ≤ 100 (from surface area increase alone, without any biotic factors) then the chemical denudation rate would be ≤ 0.01 times the present biotically enhanced rate.

We propose, therefore, that chemical weathering under biotic conditions is significantly enhanced relative to abiotic continental crust exposed to similar rainfall, temperature and p_{CO_2} conditions. The enhancement factor is uncertain; the key role of soil stabilization combined with other biotic effects suggests factors on the order of at least 100 to perhaps $>1,000$.

We now consider the implications of a present biotic enhancement of weathering ranging up to 1,000 times over the abiotic rate. Using a standard technique for quantitatively estimating effects on the carbonate-silicate geochemical cycle², we balance metamorphic and magmatic outgassing of CO_2 relative to the present, (V/V_0) , with the CO_2 sink of global silicate weathering. This weathering is a product of terms, relative to the present values, for the temperature dependence of global runoff, $e^{\gamma(\Delta T)}$, and of mineral dissolution, $e^{\beta(\Delta T)}$. The ΔT is the global average temperature deviation from the present value ($\Delta T = T - T_0$). We also include the weathering dependence on atmospheric CO_2 into a unitless term to account for the biological enhancement. The actual values for the p_{CO_2} terms are most correctly taken as soil p_{CO_2} (ref. 5), but because in the abiotic case $p_{\text{CO}_2} = p_{\text{CO}_2}^{\text{soil}}$ and $p_{\text{CO}_2}^{\text{soil},0}$ can be written as $n(p_{\text{CO}_2}^0)$ where $10 < n < 100$ (ref. 8), we can write $(p_{\text{CO}_2}^{\text{soil}}/p_{\text{CO}_2}^{\text{soil},0})^\alpha = (p_{\text{CO}_2}/p_{\text{CO}_2}^0)^\alpha (1/n)^\alpha$ and then include the constant term $(1/n)^\alpha$ in a term that accounts for the biological enhancement. Expressed as a ratio of the abiotic weathering rate, B_{abiotic} , and the rate for the present Earth, B_0 , this term is taken here as a concatenation of all the specific biota-linked processes described above. We include a land area term (A/A_0) on the weathering side of the equation³, and write:

$$\frac{V}{V_0} = \frac{B_{\text{abiotic}}}{B_0} \left(\frac{p_{\text{CO}_2}}{p_{\text{CO}_2}^0} \right)^\alpha e^{\beta(\Delta T)} e^{\gamma(\Delta T)} \frac{A}{A_0} \quad (1)$$

The biotic enhancement, B_0/B_{abiotic} , is varied between 1 and 1,000. We taken $\alpha = 0.4$ (discussed above), $\beta = 0.056$ (ref 2) and $\gamma = 0.017$ (ref. 2). We present results using three different greenhouse calculations (see Fig. 1 caption for further details). The results in Fig. 1 show that if the present, biotic enhancement ratio, B_0/B_{abiotic} , is 10, then an abiotic Earth would be 10–15 °C warmer. If the biotic enhancement is 100, the abiotic Earth would be warmer by 20–40 °C, and if 1,000, warmer by 35–60 °C. If the present enhancement $\geq 1,000$, then all greenhouse functions result in a surface temperature of greater than 50 °C ($T = T_0 + \Delta T$; $T_0 = 15^\circ\text{C}$). The temperature 50 °C is the estimate of the habitability limit for all but thermophile microbes, because of thermolability of proteins and cellular membranes of the non-thermophiles³¹. Even with a biotic enhancement ratio as low as 80, greenhouse functions of refs 2 and 32 give a surface T of 50 °C. A pioneering quantitative approach to estimating temperatures on a present abiotic Earth was made by Lovelock and Watson¹, assuming the diffusion of atmospheric CO_2 into soil. They calculated a temperature increase of $\sim 20^\circ\text{C}$ corresponding to atmospheric $p_{\text{CO}_2} = 0.01$ bar (ref. 31 gives a ΔT of

13 °C for this p_{CO_2}) or roughly the present soil p_{CO_2} , to equal volcanic emissions. Although they recognized that the p_{CO_2} and temperature would be somewhat higher because of a concentration gradient in the soil, the model did not include the temperature dependence of weathering which would lower the required p_{CO_2} and surface temperature. In any case, as we have argued, simply subtracting biotic enhancement of soil p_{CO_2} only goes part of the way to abiotic conditions.

Life appeared at least 3.6 if not 3.8 Gyr ago, with the oldest fossils occurring at 3.6 Gyr and indications from carbon isotope fractionation in metasediments that a biota (photosynthetic bacteria) existed at 3.8 Gyr (ref. 33). The most primitive extant life forms are, apparently, thermophile archaeobacteria³⁴, some enjoying optimal growth at 105 °C. If the transition from abiotic to biotic conditions occurred 3.8 Gyr ago with a transition temperature of the order of 100 °C, this required $p_{\text{CO}_2} = 15$ bars in the atmosphere (ref. 32, greenhouse function), agreeing with other estimates³⁵. Outgassing rates were possibly higher in the Archaean as a result of a greater juvenile contribution, as well as lack of subduction loss of carbon to the mantle³⁶. Kump (personal communication) suggests, however, that the Archaean outgassing rates were lower than the present rates because of the relative absence of carbonate sediments available for metamorphism. Higher chemical-weathering intensities would probably be required to balance outgassing, in any case, because of significantly smaller Archaean land areas, necessitating much higher p_{CO_2} in the atmosphere and surface temperature for abiotic models then, than for the present abiotic case.

The maximum allowable intensity of chemical weathering would be limited by global-uplift rates³⁷. If mean uplift rates in the early Archaean were significantly higher than today's rate (E. Nisbet, personal communication) then higher chemical denudation rates would be possible. Alternatively, a steady state CO_2 level in the atmosphere-ocean system was not achieved until later in the Precambrian, as volcanic emissions declined and land areas increased.

We offer the following scenario: origin of life near 100 °C, colonization of land by ancestral archaeobacteria and photosynthetic bacteria (note that water is of course liquid at 100 °C at external pressures >1.0 bars, well below the p_{CO_2} inferred for an abiotic Earth in the early Archaean); natural selection would favour mutants with greater nutrient-extraction ability and growth forms with habits promoting water retention, all which would drive up weathering rates leading to soil development and drive down p_{CO_2} and surface temperatures to allow the evolution of lower-temperature forms, the microbial biota of the Archaean. The history of surface temperatures on early Earth³⁵ is consistent with a major increase in biotic influence with the appearance of land biota in the Archaean; subsequent developments, such as emergence of vascular land plants¹⁰, and then angiosperms⁶, produced further increases. We believe this is a plausible scenario for the emergence of a geophysiological³⁸ influence on global temperature with life playing an essential part along with inorganic geological processes. The Earth's earliest biota in this scenario did not optimize conditions for its existence, but was a critical factor in creating conditions for low-temperature life to emerge and thrive. □

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⁴⁰Ar/³⁹Ar laser-probe dating of diamond inclusions from the Premier kimberlite

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INCLUSIONS encapsulated by diamonds at the time of their formation provide a means for determining diamond crystallization ages and the chemistry of the surrounding upper mantle at that time. Sm–Nd studies of peridotitic inclusions, from Cretaceous-age kimberlites in southern Africa, suggest that the diamonds formed 3.3 Gyr ago¹. By contrast, eclogite-suite inclusions generally yield younger ages, sometimes approaching the time of kimberlite eruption^{2–4}. Here we report the results of ⁴⁰Ar/³⁹Ar laser-probe analyses of individual eclogitic clinopyroxene inclusions from Premier diamonds, which yield a mean age of $1,198 \pm 14$ Myr. This age agrees well with Sm–Nd (ref. 2) and ⁴⁰Ar/³⁹Ar (ref. 3) analyses on similar Premier inclusions, and is indistinguishable from the inferred time of emplacement of the host kimberlite ($1,150$ – $1,230$ Myr)², which implies that diamond formation was essentially synchronous with kimberlite generation. The extrapolated non-radiogenic ⁴⁰Ar/³⁶Ar ratio of 334 ± 102 is similar to the present-day atmospheric composition. This value is inconsistent with Sr and Nd isotopic signatures from Premier eclogite inclusions², which suggest a depleted mantle source (⁴⁰Ar/³⁶Ar > 20,000). Pre-entrapment equilibration of the inclusions with an ³⁶Ar-rich fluid is the most probable explanation for the low non-radiogenic (⁴⁰Ar/³⁶Ar) composition.

Because of their chemical inertness and high-pressure/temperature stability at low f_{O_2} , diamonds provide a resilient refuge for impurities and inclusions ranging from atomic helium to millimetre-sized silicate grains^{5–8}. Consequently, diamonds and

their inclusions provide a unique opportunity to ascertain the mantle chemistry at the time of diamond formation. Syngenetic silicate inclusions are present in a small proportion of diamonds and can usually be classified into eclogitic or peridotitic associations⁹. The peridotite suite includes olivine, enstatite and pyrope whereas the eclogitic suite is dominated by garnet and omphacitic clinopyroxene^{10,11}. Eclogitic clinopyroxene is of particular interest to K–Ar dating of diamonds, because of their high potassium contents (≤ 0.9 wt% K₂O (ref. 12)).

So far, the most successful attempts to date diamonds by their inclusions have used the U–Pb and Sm–Nd decay schemes. Kramers¹³ reported U–Pb model ages of >2,000 Myr and ~1,200 Myr from two suites of sulphide inclusions from diamonds, the first derived from the ~90 Myr Finsch- and Kimberley-group kimberlites and the second from the ~1,180 Myr Premier kimberlite. Richardson *et al.*¹ obtained Sm–Nd model ages averaging $3,300 \pm 200$ Myr (2σ error) from peridotite-suite inclusion composites extracted from Finsch- and Kimberley-group diamonds. In a subsequent study², two-point Sm–Nd isochron ages of $1,150 \pm 60$ Myr (2σ error) for Premier and of $1,580 \pm 60$ Myr (2σ error) for the Argyle mine (Australia) eclogitic diamond inclusions were obtained. The wide range of Sm–Nd model ages, from $1,443 \pm 188$ to $2,408 \pm 180$ Myr (ref. 4), measured for large single eclogitic inclusions from the Finsch (90 Myr) kimberlite implies multiple episodes of diamond genesis.

K–Ar and ⁴⁰Ar/³⁹Ar analytical techniques have been less successful in constraining diamond formation ages. In many cases, isotope measurements have been made on whole

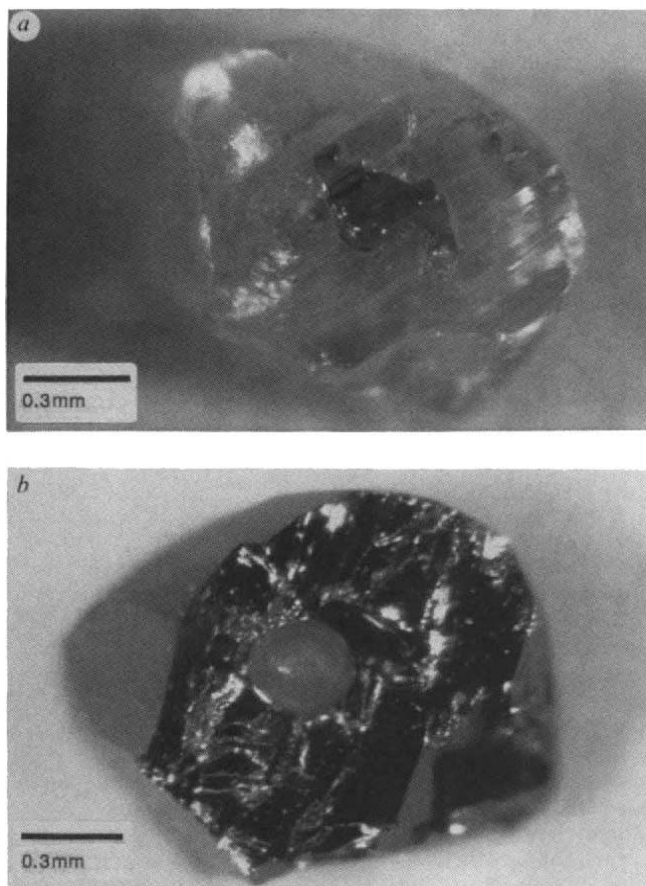


FIG. 1 a, Photomicrograph of Premier clinopyroxene inclusion P120, partly exposed in its host diamond. b, P120 after irradiation and laser-probe fusion. the diamond turned dark green after irradiation. On laser heating, the clinopyroxene inclusion coalesced to a bead above the cavity in the diamond that it had occupied previously.

diamonds, often of unknown origin and of undetermined inclusion abundance or paragenesis^{6,14,15}. Using a laser probe, Turner *et al.*¹⁶ determined an $^{40}\text{Ar}/^{39}\text{Ar}$ apparent age of $1,440 \pm 140$ Myr from a Premier-mine eclogitic garnet inclusion, but they were unable to correct for non-radiogenic Ar due to the small gas volumes released. At the same time as our work was being undertaken, Burgess *et al.* measured³ the $^{40}\text{Ar}/^{39}\text{Ar}$ laser-probe apparent ages of seven eclogitic Premier clinopyroxene inclusions ranging³ from $1,111 \pm 35$ to $1,254 \pm 38$ Myr with an average of $1,185 \pm 94$ Myr (2σ error). This range of 143 Myr may be partially attributable to problems in the accurate determination of non-radiogenic ^{36}Ar contents.

The Premier kimberlite is located in the middle of the Archaean Kaapvaal Craton, Transvaal Province, South Africa and intrudes the Bushveld Igneous Complex. Attempts to date the time of kimberlite emplacement have yielded ages ranging from 1,150 to 1,230 Myr (ref. 2). The mineralogy of Premier-diamond inclusions is well documented¹⁰. Eclogitic-suite inclusions are more abundant than peridotite-suite inclusions by a margin of 3:2 and include sulphides, pyrope-almandine garnet, omphacitic clinopyroxene and rare kyanite and coesite.

Here, ten eclogitic clinopyroxene inclusions, ranging from $250 \times 200 \times 100 \mu\text{m}$ to $700 \times 200 \times 200 \mu\text{m}$, were separated from Premier diamonds. Inclusion P120 was provided protruding from its host diamond and was analysed *in situ* (Fig. 1). $^{40}\text{Ar}/^{39}\text{Ar}$ measurements were taken with the Princeton University Continuous-Laser Argon-Age Microprobe (CLAAMP), which uses a Quantronix 117 Nd YAG laser, operated in continuous mode. The samples are contained in a stainless-steel holder beneath a sapphire window. The released gas is purified in a system of getters and an alcohol/dry-ice stainless-steel cold trap. The isotope analyses were carried out on a Micromass 1200S mass spectrometer with a Bauer-Signer ion source and a Johnston multiplier detector. A more detailed account of the laser-probe analytical techniques and sample preparation

methods are given elsewhere (ref. 17). In particular, attention was paid to the accurate determination of the ^{36}Ar concentration of the inclusions; each analysis of either the blank or the sample involved 28 measurements of the ^{36}Ar signal compared to 7 measurements for the other isotopes. The argon-isotope results for the clinopyroxene diamond inclusions, Mmhb-1 hornblende standard grains and the mean blank levels are presented in Table 1. All uncertainties quoted are two standard deviations. Because of the variations in inclusion size and potassium content, some inclusions were analysed individually, but others were combined to provide sufficient argon for accurate measurement (Table 1). A two-stage step-heating schedule was employed for inclusion P121.

All the clinopyroxene inclusions had very low (but nevertheless measurable) ^{36}Ar and high ^{39}Ar contents, consistent with expected quantities of K (Table 1). The range of K_2O estimates derived from $^{37}\text{Ar}_{\text{Ca}}/^{39}\text{Ar}_{\text{K}}$ ratios¹⁹, 0.03 ± 0.01 – 0.31 ± 0.09 wt%, is consistent with the diamond-inclusion electron-microprobe results. Inclusion P121, which was subjected to a two-stage heating schedule, was dated at $1,161 \pm 96$ Myr (low-temperature step) and $1,206 \pm 22$ Myr (fusion step); these ages agree within the uncertainty. Infrared radiance measurements indicate temperatures of 900–1,000 °C for the first step and >1,400 °C for the fusion step.

Apparent ages range from $1,159 \pm 70$ to $1,207 \pm 22$ Myr if calculated on the assumption that the non-radiogenic Ar has an atmospheric $^{40}\text{Ar}/^{36}\text{Ar}$ composition of 295.5. Because this assumption may not be justified, the $^{36}\text{Ar}/^{40}\text{Ar}$ contents were plotted against the $^{39}\text{Ar}/^{40}\text{Ar}$ contents (Fig. 2). Although the $^{36}\text{Ar}/^{40}\text{Ar}$ values lie close to the x-axis, they form a distinct linear trend towards lower $^{39}\text{Ar}/^{40}\text{Ar}$ and higher $^{36}\text{Ar}/^{40}\text{Ar}$ values. This relationship is in accordance with the mixing of a radiogenic-rich with a non-radiogenic argon component²⁰. The application of a weighted least-squares routine²¹ to the data gave an x-intercept corresponding to an age of $1,198 \pm 14$ Myr

TABLE 1 $^{40}\text{Ar}/^{39}\text{Ar}$ laser-probe analytical results for eclogitic clinopyroxene inclusions from diamonds recovered from the ~1,180 Myr old Premier kimberlite, South Africa

Sample	Estimated mass (μg)†	Estimated K_2O (wt%)‡	^{36}Ar §	^{37}Ar	^{38}Ar ($\times 10^{-12} \text{ cm}^3$ at STP)	^{39}Ar	^{40}Ar	$^{37}\text{Ar}_{\text{Ca}}/^{39}\text{Ar}_{\text{K}}$	Apparent age (Myr)
Clinopyroxene inclusions									
P117	16.99	0.12 ± 0.06	0.06	173.7	0.10	3.33	127.1	53.9 ± 2.6	$1,154.5 \pm 69.6$
P118/9¶	49.46	0.07 ± 0.04	0.14	505.1	0.12	5.93	224.1	90.2 ± 1.2	$1,193.7 \pm 29.2$
P120	30.37	0.31 ± 0.18	0.19	287.9	0.25	13.78	578.2	21.2 ± 0.2	$1,197.4 \pm 23.2$
P121A (low T)	92.61	0.33 ± 0.18	0.02	55.4	0.02	2.79	105.7	20.1 ± 1.8	$1,161.3 \pm 95.6$
P121B (fusion)	—	0.31 ± 0.18	2.28	821.9	5.50	39.23	1,558.1	21.2 ± 0.2	$1,206.1 \pm 22.2$
P122/3	37.84	0.03 ± 0.02	1.11	359.1	1.12	2.03	74.9	200.4 ± 13.4	$1,164.2 \pm 91.6$
P124/6	15.36	0.15 ± 0.08	0.04	145.7	0.06	3.45	130.8	43.4 ± 1.0	$1,175.2 \pm 45.8$
Mmhb-1 hornblende (irradiation standard)									
Mean of 9 grains:	—	—	0.11	146.0	1.45	56.94	831.3	2.56 ± 0.24	523.5 ± 10.0
$J = 0.02373 \pm 0.000237$									

Samples were packed in a cadmium-lined aluminium canister, with packets of irradiation standard Mmhb-1²⁹, and irradiated at McMaster University reactor, Hamilton, Ontario.

† Masses (μg) estimated from ^{39}Ar volumes and K_2O contents.

‡ K_2O contents estimated from $^{37}\text{Ar}_{\text{Ca}}/^{39}\text{Ar}_{\text{K}}$ values: Ca/K ratios calculated from $^{37}\text{Ar}_{\text{Ca}}/^{39}\text{Ar}_{\text{K}}$ ratios using the conversion factor of $1.82(\pm 0.17)$ from ref. 19; K contents were calculated using average Ca values from ref. 10. Errors (1σ) include uncertainties in microprobe analyses and conversion factor.

§ $^{36}\text{Ar}/^{40}\text{Ar}$ abundances ($\times 10^{-12} \text{ cm}^3$ at STP) are corrected for system blanks and decay of ^{37}Ar and ^{39}Ar , but not for interference reactions from Ca, K and Cl. Blank levels and errors for sample runs were determined from linear least-squares regressions of 2–4 blank runs before and after each sample. Mean blank levels and errors were: 2.8 – $4.5 \times 10^{-13}(\pm 3.5\%) \text{ cm}^3$ at STP for ^{36}Ar , 1.6 – $2.0 \times 10^{-12}(\pm 1.6\%) \text{ cm}^3$ at STP for ^{37}Ar , 2.8 – $5.0 \times 10^{-13}(\pm 3.9\%) \text{ cm}^3$ at STP for ^{38}Ar , 1.4 – $1.9 \times 10^{-12}(\pm 2.1\%) \text{ cm}^3$ at STP for ^{39}Ar and 0.6 – $1.2 \times 10^{-11}(\pm 5.3\%) \text{ cm}^3$ at STP for ^{40}Ar . For age calculations, blank- and decay-corrected peak heights were also corrected for mass discrimination, isotopic interferences¹⁸, flux gradients and atmospheric contamination assuming $^{40}\text{Ar}/^{36}\text{Ar} = 334$.

|| Apparent age (Myr) = age calculated using the decay constants. $\lambda_e = 0.581 \times 10^{-10} \text{ yr}^{-1}$; $\lambda_\beta = 4.961 \times 10^{-10} \text{ yr}^{-1}$; $^{40}\text{K}/\text{K} = 0.01167 \text{ atom\%}$; ($^{40}\text{Ar}/^{36}\text{Ar}$) = 334 for inclusions and 295.5 for Mmhb-1 grains. Quoted errors are two standard deviations and include errors in blank correction, interference corrections and J-value.

¶ Two inclusions fused simultaneously.

Weighted mean of three fusions ($\pm 20 \text{ mg}$) of irradiation standard Mmhb-1 hornblende using the Lindberg extraction system¹⁹ and the K–Ar age of $520.4 \pm 1.7 \text{ Myr}$ (ref. 29).

(mean square of weighted deviates = 0.9174; Fig. 2). Inverting the y-axis intercept gives an $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of 334 ± 102 , which is indistinguishable from the present-day atmospheric composition. Recalculation of ages for the individual inclusion analyses, using a non-radiogenic $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of 334, yields similar values, ranging from $1,155 \pm 70$ to $1,206 \pm 22$ Myr (Table 1).

The results presented in this study indicate that the laser microprobe can accurately measure argon-isotope signatures of individual diamond inclusions, provided they are of sufficient size and potassium content. This capability avoids problems of sample heterogeneity in multi-inclusion analyses and can evaluate potential differences in diamond formation ages and/or initial ($^{40}\text{Ar}/^{36}\text{Ar}$)_i ratios. The labour involved in sample selection and inclusion separation is less than that for Sm-Nd analyses, which often require bulk composites of 100–300 cogenetic inclusions^{1,2}.

The $1,198 \pm 14$ Myr age is indistinguishable from the inferred time of kimberlite emplacement¹⁷ and similar to the $^{40}\text{Ar}/^{39}\text{Ar}$ (ref. 3), U-Pb (ref. 13) and Sm-Nd (ref. 12) inclusion ages determined previously. These results are also consistent with nitrogen aggregation states in Premier diamond² which indicate mantle residence times between formation and eruption ≤ 1 –10 Myr. The Premier diamonds, therefore, appear to be xenocrysts, formed just before the kimberlite eruption.

Isotope studies also yield information about mantle conditions at the time of diamond crystallization. The Premier eclogitic inclusions exhibit higher initial Nd and lower initial Sr ratios than bulk Earth² and are characteristic of the mid-ocean-ridge basalt (MORB) mantle reservoir. MORBs are generally considered to host Ar with high $^{40}\text{Ar}/^{36}\text{Ar}$ ratios ($\sim 20,000$) (ref. 22), because of early degassing and subsequent build-up of radiogenic ^{40}Ar . The low ($^{40}\text{Ar}/^{36}\text{Ar}$)_i ratio measured from the Premier inclusions in this study is obviously inconsistent with a MORB source (Fig. 2).

One possible explanation is that Ar solubility in eclogitic inclusions is low and easily masked by contaminating modern atmospheric argon. Pre-analysis bake-out, however, should remove this component. Low-temperature atmospheric contamination is also unlikely to account for the ^{36}Ar released at the high-temperature step of inclusion P121. Similar low $^{40}\text{Ar}/^{36}\text{Ar}$ ratios, ranging from 295 ± 7 to 433 ± 40 , have been reported for inclusion-free Premier diamonds^{6,8}. Therefore, we interpret the low $^{40}\text{Ar}/^{36}\text{Ar}$ ($<1,000$) ratios as characteristic of mantle conditions prevailing at the time of Premier eclogitic diamond formation.

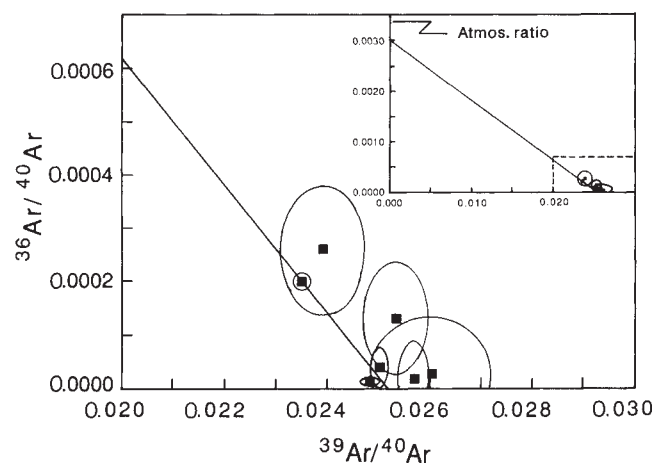


FIG. 2 A plot of $^{36}\text{Ar}/^{40}\text{Ar}$ against $^{39}\text{Ar}/^{40}\text{Ar}$ for eclogitic clinopyroxene inclusions from Premier-mine diamonds. Uncertainty ellipses represent uncertainties of one standard deviation. Intercept on the x-axis corresponds to an age of $1,198 \pm 6$ Myr and the y-intercept (inset) yields an ($^{40}\text{Ar}/^{36}\text{Ar}$)_i ratio of 334 ± 51 .

If the Premier diamonds did indeed incorporate argon with low ^{40}Ar , the Sr- and Nd-depleted eclogitic minerals must have equilibrated with ^{36}Ar -rich fluids from either a primordial, undegassed (perhaps lower) mantle²³ or from atmospheric volatiles recycled into the mantle. The former hypothesis requires transportation of noble gases from an undegassed mantle to the depleted lithosphere, with limited exchange with MORB gases, to yield a $^{40}\text{Ar}/^{36}\text{Ar}$ value indistinguishable from the present-day atmosphere. The alternative hypothesis requires a mechanism for carrying atmospheric Ar into the mantle, such as subduction of ocean crust that has been exposed to sea water. Such a model would be compatible with recent arguments that relate diamond formation and kimberlite genesis to subduction processes^{24–26}. Because sea water is characterized by high $^{87}\text{Sr}/^{86}\text{Sr}$ ratios²⁷, however, exchange with fluids from altered oceanic crust should lead to enrichment in Sr isotopes. Although this is inconsistent with the results of Richardson², high $^{87}\text{Sr}/^{86}\text{Sr}$ has been reported for eclogite garnet inclusions from Finsch diamonds⁴. Apparent discrepancies in the inferences drawn from our Ar results and Richardson's² Sr results may therefore reflect either a heterogeneous diamond inclusion population or decoupling of Ar and Sr systems. Given the high mobility of the noble gases, the latter may be readily achieved.

The results from other kimberlites do not require decoupling of Ar and Sr-Nd systematics. As stated previously, initial Sr and Nd ratios from peridotite diamond inclusions from Finsch- and Kimberley-group diamonds suggest an origin from an enriched mantle source¹. Both inclusion-free and inclusion-bearing (probably peridotitic) diamonds from these localities yield $^{40}\text{Ar}/^{36}\text{Ar}$ values ranging from 259 ± 56 to 381 ± 64 (refs 9, 28).

The diamonds appear to have grown in environments with a variety of Ar isotopic compositions, the cause of which is often ambiguous. Future isotope studies on carefully characterized diamonds and inclusions, utilizing Sr, Nd, Pb and all noble-gas systems, will certainly improve our present understanding and constrain models for diamond formation and perhaps, eventually, mantle evolution. □

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Mate choice on fallow deer leks

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LEKS, on which males defend small clustered mating territories, may have evolved because of the unusual opportunities they provide for female choice of mating partners¹⁻⁴, and several studies of lek-breeding animals have demonstrated correlations between the mating success of males and their phenotype or behaviour⁵⁻⁷. However, these could arise because (1) females select mates on the basis of male phenotypic traits¹⁻⁴; (2) males interfere with each other's mating attempts^{4,8,9}; or (3) females show preferences for particular mating territories, and larger or stronger males are more likely to win access to these territories^{4,10-12}. Here we report that when fallow bucks on a traditional lek were experimentally induced to change their territories, differences in the mating success of bucks persisted, whereas differences in the position of their territories relative to the centre of the lek did not. The observation that bucks rarely interfered with their neighbours' harems and females moved freely between bucks suggests that females choose their mates on the basis of male phenotype rather than territory type or location. In this population, the immediate factor affecting the movements of females between males was the size of a buck's harem.

In large populations of fallow deer, mature males commonly defend small mating territories, 15 to 20 metres in diameter, on traditional mating grounds or leks, which are visited by receptive females^{7,12,13}. Bucks collect groups of up to ten or more does, which we refer to as harems. Does move frequently between bucks, seldom staying with the same buck for more than a few hours. Although some of these moves appear to be voluntary, many are caused by the incursions of young bucks, who run into harems, scattering their members⁷. The majority of does come into full oestrous 1-12 h after joining the lek. Does mate once with a single buck and less than 5% mate with a second buck before leaving the lek to rejoin feeding herds, usually within 2 h of mating⁷.

Differences in mating success between bucks are large: in each of three mating seasons (15-30 October, 1986, 1987 and 1988), the most successful buck in our study population (Fig. 1) mated with ~15% of the 200 does that were seen to mate on the lek in each year, whereas ~50% of mature bucks holding territories on the lek failed to mate. Differences in mating success among territorial bucks were correlated with their average harem

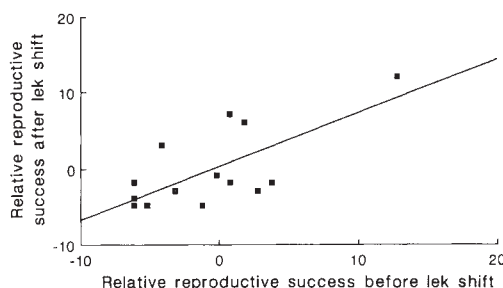


FIG. 2 Relative reproductive success (copulations per buck per day minus the mean number of copulations on that day, calculated across all territorial bucks and averaged across days) of 17 fallow bucks before and after they were induced to change territories (Kendall's $\tau=0.456$, $P<0.05$). Only 14 values are shown, as values for three pairs of bucks coincided exactly.

size and fighting success, as well as with their antler size and hind foot length⁷. As in other populations¹², does consistently preferred some territories to others and more mated on territories surrounded by others (central) than on territories on the edge of the lek⁷. Because large successful bucks were more likely to hold central territories⁷, correlations between phenotype and mating success could have been a result of the preference of does for particular territories.

To distinguish between choice of mating territories and partners we moved bucks from their usual territories. Between 15 and 22 October 1988, we allowed does to select their mating partners freely on the lek, measuring the mating success of all 20 territorial bucks. On 23 October, we pinned sheets of black polythene ground cover (10 m²) on the territories of the six most successful bucks and, on each night between 22 and 26 October, additional sheets were pinned on the territories of the three most successful bucks on each day. All sheets were removed on 27 October. A continuous record of the harem size and mating success of different bucks was maintained during daylight hours over the entire period.

Bucks whose territories had been treated initially remained on or close to their territory, but treated territories were avoided by does. Of the bucks whose territories were treated in this way, 78.6% moved to new territories within 24 h and 92.5% within 48 h. A further nine bucks moved to new territories immediately after a neighbour's territory had been treated, although four to six mature bucks remained on the original site of the lek throughout the mating season. Black sheets caused the focus of mating activity to shift 200 m to the east of the traditional lek site on 23 October and a further 100 m east on 24 October. Subsequent treatments caused it to move 400 m in the opposite direction.

FIG. 1 The traditional fallow deer lek in Petworth Park, Sussex. Around 20 mature bucks hold small territories on the lek, where they are visited by females in, or close to, oestrous. Of all copulations observed in the Park ($n=650$, over a period of 3 yr), 94% occurred on the lek. Young bucks can be seen standing between territories on the lek.



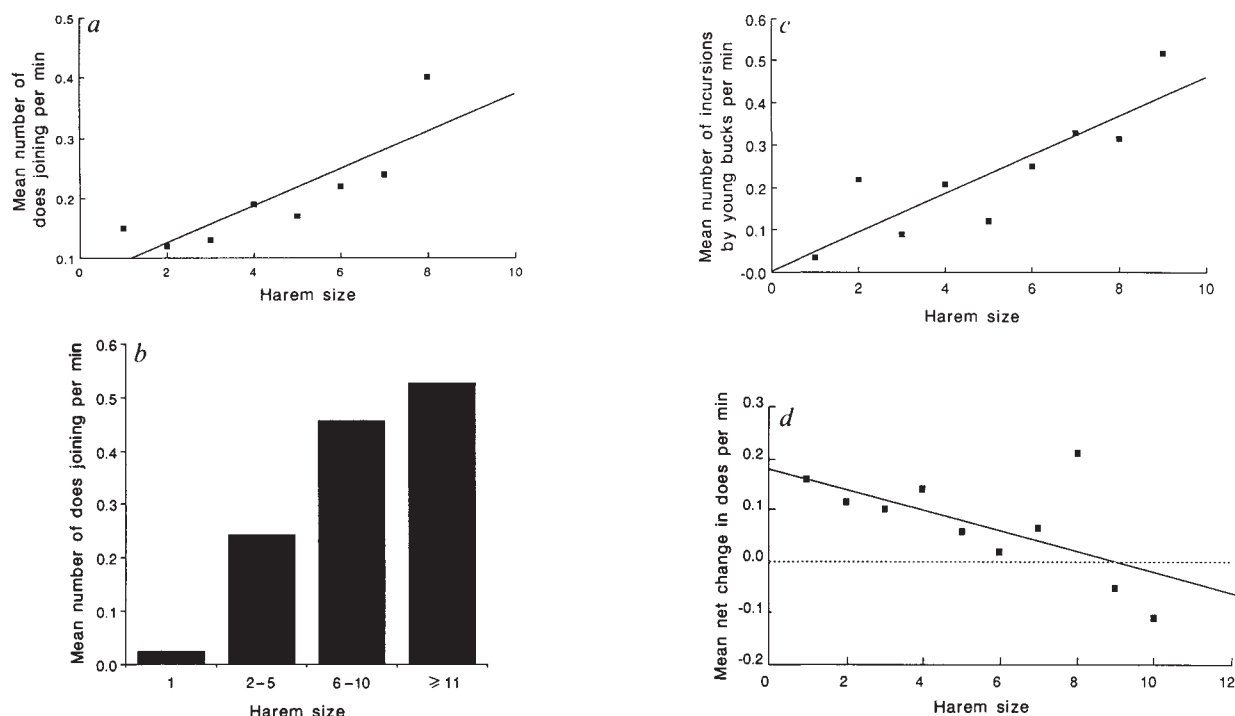


FIG. 3 *a*, Average rate at which harems of different sizes were joined by additional does, based on continuous records of all harems entered by 47 does observed from their entry onto the lek for up to 2 h (Kendall's $\tau=0.786$, $P<0.01$). Harem size was defined as the number of does present in a buck's territory at a given time. *b*, Analysis restricted to 10 bucks holding harems of each size category on the same day. Only one observation of

each buck with a harem in each size category was included in the analysis. (Friedman test for randomized blocks, $n=10$, coefficient of variation=3, $P<0.01$). *c*, Rate of incursion by young bucks into harems of different sizes (Kendall's $\tau=0.722$, $P<0.01$). *d*, Net rate of change in harems of different sizes. (Kendall's $\tau=-0.511$, $P<0.01$). Samples for *c* and *d* as for *a*.

For 17 bucks that changed territories because of the treatment, there was a significant correlation between measures of mating success (average harem size, total matings and mating success relative to the daily average) when on their original territories with those when on fresh territories (Kendall's $\tau=0.426$, 0.441 , 0.456 respectively, $P<0.05$; see Fig. 2). Although the correlation was strengthened by the persistent preference of the does for the most successful buck, the rank correlation coefficient was still significant when this buck was removed from the analysis (Kendall's $\tau=0.337$, $n=16$, $P<0.05$). By contrast, there was no significant relationship between the proportion of time for which individual bucks held central territories before versus after treatment (Kendall's $\tau=0.267$, $n=17$).

The ability of some bucks to maintain their mating success on different territories was remarkable. For example, the most successful buck initially held a central territory on the original lek site, where he mated with 17 does between 15 and 22 October. After his territory was treated on 22 October, he moved 200 m to the east to a peripheral territory, and mated with a further four does on 23 October. After this territory was treated the following day, he moved 100 m further east to another peripheral territory, mating with six more does on 24 October. When this territory received a black sheet, he moved 400 m back to the far side of the original lek and mated once on 26 October, and when this territory was treated he moved a further 50 m west, again to a peripheral territory, and mated with five more does on 27 October. Does moved freely between territories, and territorial bucks rarely interfered with each other's harems. Also, all territories were located on similar vegetation (long *Agrostis* grassland, mixed with *Juncus*) and the resources on lek territories have been shown not to affect the distribution of females⁷. These observations consequently suggest that persistent differences in harem size and mating success between bucks were caused by female preferences for some characteristic of males.

There was no significant tendency, however, for does to move to larger bucks or to those with larger antlers. The main criterion

affecting the movements of does between bucks seemed to be the size of a buck's harem. Does joining the lek consistently entered harems of above average size (Kolmogorov-Smirnov one sample test, $D=36.439$, $n=18$, $P<0.01$) and spent more time in the first territory selected if there were already several does in it (Kendall's $\tau=0.850$, $n=16$, $P<0.05$). In successive moves between territories, does also favoured larger harems over smaller ones, so that the rate at which harems attracted additional does increased with their size (Fig. 3*a*). This was unlikely to have occurred because does independently selected bucks on some physical criterion, as the frequency with which the same bucks attracted does increased with changes in their harem size on the same day (Fig. 3*b*). Harems did not continue to increase in size indefinitely because the rate of incursions by young bucks (who drive does out of territories) also rose with harem size (Fig. 3*c*) so that harem size usually declined after a buck had eight does in his territory (Fig. 3*d*).

These results suggest that fallow does choose their mates on the basis of their phenotype, although territory position may also influence mate choice. Female movements between harems seem to be governed by the distribution of does already on the lek, but the tendency for does to return to the same bucks when the lek is temporarily cleared indicates that other factors must be important. These may be difficult to identify unless it is possible to examine the behaviour of the first females visiting a lek. □

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DNA phylogeny of the extinct marsupial wolf

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THE phylogenetic affiliation of the extinct marsupial wolf (*Thylacinus cynocephalus*), which once was widespread in Australia, has been uncertain. On the basis of morphology, some systematists argue that the thylacine was most closely related to an extinct group of South American carnivorous marsupials, the borhyaenids^{1–3}, whereas others consider it to be closer to Australian carnivorous marsupials⁴. Here we use direct sequencing by means of the polymerase chain reaction (PCR) to compare 219 bases of mitochondrial (mt) DNA from museum specimens of the marsupial wolf and representatives of six genera of extant marsupials. In agreement with the results of an antigenic study of albumin⁵, our genetic data suggest that the marsupial wolf was more closely related to other Australian marsupial carnivores than to those of South America. Thus, the marsupial wolf represents an example of convergent morphological evolution to South American carnivorous marsupials as well as to true wolves.

Direct sequencing by means of the PCR^{6,7} has enabled the retrieval of DNA sequences from museum specimens and archaeological remains^{8–11}. This is because the PCR can synthesize many copies of a small number of intact DNA molecules in the presence of a vast excess of damaged molecules⁹. To perform the PCR on thylacine DNA, we designed primer pairs that matched sequences in the genes for cytochrome *b* and the 12S ribosomal (r) RNA in the mtDNA of other vertebrates¹². Each primer pair was selected so that it would amplify a fragment of, at most, 160 base pairs (bp), because longer sequences of old DNA have generally proved impossible to amplify^{8,9}. We performed extractions of thylacine DNA from several samples (0.1 g) of tissue. One hide sample yielded a human mtDNA sequence, whereas a muscle sample gave a double sequence of both marsupial and human origin. Amplification of shorter sequences (~80 bp), however, from the muscle sample gave exclusively marsupial sequence. Human contamination is a frequent problem when amplifying sequences from old specimens, particularly if they have been tended for a long time by museum curators. Furthermore, DNA stemming from the specimen itself is usually more degraded and/or damaged than the contaminating DNA. This results in the specimen sequence becoming more predominant in the amplification products as the amplified sequences become shorter¹⁰. The preparation from a second hide sample gave unambiguous marsupial sequences

of up to 160 bp, whereas amplifications of longer sequences yielded no specific products.

Figure 1 shows the sequences of the 12S rRNA and cytochrome *b* gene segments for the thylacine and six other marsupials. Through alignment by eye we found 94 bp of each of the 12S rRNA sequences that could be used for phylogenetic analyses. Table 1 shows the numbers of transversion and transition differences for each pair of species. For the 12S sequence the numbers of transversions separating the tested marsupials and the placental mammal (cow) exceed those separating the marsupials from one another. Transitions in the 12S gene show a less pronounced tendency for an increase correlated with increasing phylogenetic distance. This implies that saturation (due to multiple substitutions at the same site) is being approached for transitions in the 12S rRNA gene^{13,14}. For the cytochrome *b* sequences most of the changes are at silent sites, where transversions as well as transitions seem to have reached saturation. Therefore, the cytochrome *b* sequence seems to evolve faster than the 12S rRNA sequence in marsupials. This is in agreement with our observations for mtDNA of placental mammals, where the segment of cytochrome *b* analysed evolves at least five times as fast as this 12S rRNA gene segment (data not shown). For this reason, only the 12S rRNA sequences were used for the phylogenetic tree analysis.

The tree shown in Fig. 2 relates the marsupials to one another based on the most parsimonious accounting for the differences observed in the 12S rRNA sequences. A tree in which the thylacine represents an early radiation with respect to Australian marsupials, comparable to that of the South American opossum (*Philander*), would require five more evolutionary substitutions. In a statistical test of the branching order of the tree shown in Fig. 2 (see legend), the thylacine forms a group with the other Australian carnivorous marsupials 98% of the time. Thus, the marsupial wolf falls well within the radiation of Australian marsupials, where it is closely related to the dasyurids, a group that includes the Tasmanian devil and Australian tiger cat.

The close relationship of the 12S rRNA gene in the marsupial wolf to that in dasyurids receives support from the sequences of the cytochrome *b* gene. Among these three species the transition bias typical of mtDNA can be discerned (Table 1), whereas for most of the other species the record of the transition bias has been erased by multiple substitutions. This indicates that the dasyurids and the thylacine diverged more recently than the other species studied^{13,15}.

A rough estimate of the time of divergence of the thylacine lineage from that leading to the dasyurids can be made from the average percentage of substitutions that are transitions (Table 1). This value (~70%) is similar for both the cytochrome *b* and the 12S rRNA genes and places the divergence of these species at 10–20 million years (Myr) ago, which is the time required to drop halfway to the plateau value of transitions (45%)^{13,15}. Such a date is earlier than that inferred from

TABLE 1 Pairwise comparisons of transversions/transitions in marsupial mtDNAs

mtDNAs compared	Thyl.	Sarc.	Dasy.	Echy.	Tric.	Phal.	Phil.	Bos
<i>Thylacinus</i>	—	2/4	1/3	6/5	4/7	5/6	5/6	14/11
<i>Sarcophilus</i>	9/16	—	1/1	6/4	4/8	5/7	5/5	14/11
<i>Dasyurus</i>	6/16	3/11	—	5/5	3/7	4/7	4/6	13/12
<i>Echymipera</i>	10/14	11/15	10/11	—	2/5	1/6	5/1	10/9
<i>Trichosurus</i>	11/14	12/15	11/14	11/17	—	1/3	3/5	12/12
<i>Phalanger</i>	11/13	14/16	13/12	13/18	8/13	—	4/7	11/13
<i>Philander</i>	9/13	12/13	9/10	7/16	12/15	14/13	—	9/10
<i>Bos</i>	12/11	13/15	12/11	12/14	11/15	15/15	11/11	—

The numbers of transversion differences followed by the numbers of transition differences are given above the diagonal for the 94 bp of the 12S rRNA gene and below the diagonal for the 116 bp segment of the cytochrome *b* gene. A transversion is the replacement of a pyrimidine by a purine or vice versa; a transition is the replacement of a purine by a different purine or a pyrimidine by a different pyrimidine.

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cluster of marsupial lineages contrasts with the score of zero to five against it derived from the molecular results⁵ (Fig. 1). This statistically significant discrepancy justifies the proposal that the dental and pelvic traits shared uniquely by thylacines and borhyaenids suggest a remarkable amount of convergent or parallel evolution, resulting in the resemblance between these species. The marsupial wolf is thus a striking example of morphological convergence not only to placental wolves but also to South American carnivorous marsupials. This is likely to have been caused by parallel adaptations to similar modes of predation on different continents. The study of the molecular basis for such convergence at the level of the organism is a challenge for biology. □

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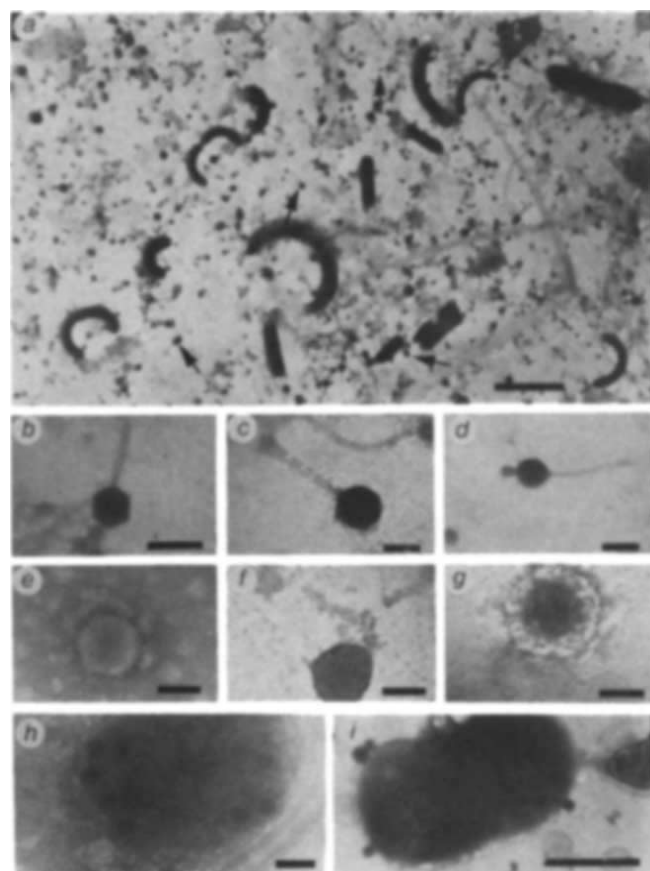


FIG. 1 Phages and bacteria observed in water samples from various natural marine and limnic ecosystems. *a*, Overview of bacteria and virus-sized particles from Lake Plussee. *b–e*, Particles classified as bacteriophages according to size and morphology. *f–g*, Particles of uncertain affiliation, but classified as viral particles in total counts. *h*, Bacterium with virus-like particles inside. *i*, Bacterium with phages attached to its surface. The particles, including bacteria, were collected by centrifugation of fixed water samples, 2% glutaraldehyde (Plussee, Chesapeake Bay, North Atlantic), 2% formaldehyde (Barents Sea), or from unfixed samples (Korsfjorden, Raunefjorden). Water samples were filled in centrifuge tubes with plastic-moulded flat bottoms. Grids with carbon-coated formvar film were then dropped onto the flat bottom and the samples were centrifuged (swing-out rotor) at 100,000g for 90 min. After removal of water, the grids were air-dried, stained with uranyl acetate (2%) and total bacteria and virus particles counted at high magnification ($\times 20,000$ – $100,000$) in a Jeol 100-CX transmission electron microscope. Scale bars: *a*, 1 μm ; *b–d* and *h*, 0.1 μm ; *e–g*, 0.05 μm ; *i*, 0.5 μm .

High abundance of viruses found in aquatic environments

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THE concentration of bacteriophages in natural unpolluted waters is in general believed to be low^{1,2}, and they have therefore been considered ecologically unimportant³. Using a new method for quantitative enumeration, we have found up to 2.5×10^8 virus particles per millilitre in natural waters. These concentrations indicate that virus infection may be an important factor in the ecological control of planktonic micro-organisms, and that viruses might mediate genetic exchange among bacteria in natural aquatic environments.

The highest total counts of viruses and bacteria were found in samples from the eutrophic lake Plussee (Table 1). We found total counts of viruses of between 5×10^6 and 15×10^6 per ml in marine samples taken during the productive part of the year. Marine samples taken in winter, however, were found to have very low numbers of viruses (Table 1), thus indicating a seasonal variation in the concentration of viruses in natural waters. Our virus counts are 10^3 – 10^7 times higher than previous reports on virus numbers in natural aquatic environments, which are based

on counts of plaque-forming units using various host bacteria^{1,2}. Previously, most marine bacteriophages that have been isolated and described have had a head size larger than 60 nm (ref. 4). We have found, however, that smaller viruses with head size less than 60 nm seem to dominate natural populations (Table 1).

Bacteriophages that can be assigned to the Bradley groups A or B⁵ are easily recognized by their tail structures (Fig. 1*a* (arrowheads), *b*, *c* and *d*). Some examples of virus-like particles, apparently without any tail structure but otherwise of similar size and morphology, are shown in Fig. 1*e*, *f* and *g*. These particles may be bacteriophages of Bradley groups C, D or E; or they may be phages of group A or B that have lost their tail. They may also be viruses relating to eukaryotic hosts such as microalgae.

Most of the virus particles we observed appeared to be free in the water, but some were also associated with bacteria. The bacterium shown in Fig. 1*h* seems to be in a phase of lytic disruption with numerous virus-like particles inside. Figure 1*i*

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TABLE 1 Total numbers of virus particles and bacteria in samples from various locations

Location	Sampling depth (m)	Sampling date	Bacteria	Numbers of virus and bacteria (10^6 ml^{-1})				Total number of viruses
				30–60 nm	Virus head size 60–80 nm	80–100 nm	>100 nm	
Plussee, West Germany	0.2	24 May 1988	6.2	166	43.9	36.6	7.3	254
Chesapeake Bay 38°33.5'N, 76°18.5'W	1	27 May 1988	3.2	4.1	2.5	2.5	1.0	10.1
Korsfjorden 60°11.7'N, 5°12.5'E	1	5 October 1988	1.1	2.4	1.6	1.5	0.6	6.1
Raunefjorden 60°16.2'N, 5°12.5'E	1	10 August 1988	0.2	6.1	3.0	0.8	ND	9.9
Raunefjorden 60°16.2'N, 5°12.5'E	1	1 February 1989	0.5	ND	ND	ND	ND	<0.01
Raunefjorden 60°16.2'N, 5°12.5'E	1	13 March 1989	0.5	1.9	1.1	1.2	0.6	4.8
North Atlantic 48°50.1'N, 16°30.8'W	10	8 May 1988	0.3	10.3	4.1	0.2	0.3	14.9
Barents Sea 71°30'N, 31°13'E	30	25 January 1989	0.02	0.02	0.02	0.02	ND	0.06

ND: not detectable (detection limit: 10^4 particles per ml).

shows a cell apparently being infected; several viruses can be seen attached to its surface.

It is often assumed that bacterial production is maintained in balance by protozoan grazing. But recent work^{6–9} has suggested that grazing is not always sufficient to explain the mortality of bacteria in aquatic ecosystems. To assess the possible importance of bacteriophages for bacterial mortality in natural waters, we have considered theoretically the rate of phage adsorption. Assuming a population density of bacteria and of phages of 10^6 and 10^7 per ml, respectively, and the formation of 100 different phage–host systems, each containing 1% of the two populations, a first-order equation can be used to describe the adsorption of bacteriophages to host cells¹⁰. It is possible to calculate that for each of these phage–host systems the rate of phage adsorption is $2.5 \text{ min}^{-1} \text{ ml}^{-1}$, assuming the adsorption-rate constant determined for T4 phages ($0.25 \times 10^{-8} \text{ cm}^3 \text{ min}^{-1}$) (ref. 10) is valid also for marine phages. The total rate of phage adsorption will then be $250 \text{ min}^{-1} \text{ ml}^{-1}$, corresponding to $3.6 \times 10^5 \text{ day}^{-1} \text{ ml}^{-1}$. Thus, as much as one-third of the bacterial population may experience a phage attack each day. If each attack results in infection and phage production, and the burst size is assumed to be 50, the rate of phage production will be $1.8 \times 10^7 \text{ phages day}^{-1} \text{ ml}^{-1}$. We conclude that the measured concentration of bacteriophages and host cells in natural waters is high enough for phages to be of quantitative importance. If the phages are temperate rather than virulent, phage production will not depend on the infection rate or the concentration of phage–host systems, but on the environment factors inducing phage production and cell lysis.

A high phage–host interaction rate in natural waters implies the possibility of active transfer of genetic information between host populations subjected to infection by the same phage strain. Thus, it is possible that genes may spread from genetically engineered microorganisms introduced into the environment to the indigenous bacterial population.

Our approach for total counting of viruses may prove valuable to establish their role in the ecology of aquatic microorganisms. The interpretation of the total virus counts will, however, have to rely on studies of virus proliferation rates, and on studies of the organisms parasitized by these viruses. The figures we have obtained from coastal, open ocean and freshwater systems suggest that the importance of the viral populations should not be neglected in any aquatic environment. □

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Function of identified interneurons in the leech elucidated using neural networks trained by back-propagation

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MECHANICAL stimulation of the body surface of the leech causes a localized withdrawal from dorsal, ventral and lateral stimuli. The pathways from sensory to motor neurons in the reflex include at least one interneuron¹. We have identified a subset of interneurons contributing to the reflex by intracellular recording, and our analysis of interneuron input and output connections suggests a network in which most interneurons respond to more than one sensory input, most have effects on all motor neurons and in which each form of the behaviour is produced by appropriate and inappropriate effects of many interneurons. To determine whether interneurons of this type can account for the behaviour, or whether additional types are required, model networks were trained by back-propagation² to reproduce the physiologically determined input–output function of the reflex. Quantitative comparisons of model and actual connection strengths show that model interneurons are similar to real ones. Consequently, the identified subset of interneurons could control local bending as part of a distributed processing network in which each form of the behaviour is produced by the appropriate and inappropriate effects of many interneurons.

The main input to the local bending reflex (Fig. 1a) is provided by four pressure-sensitive mechanosensory neurons (P cells)

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that respond selectively to dorsal or ventral touch (Fig. 1*b*)³. Contraction and relaxation of longitudinal muscles is produced by eight types of motor neurons which excite and inhibit the dorsal and ventral muscles on each side of the animal^{4,5}. The input-output function of the reflex was determined by recording intracellular signals from motor neurons of each type during single or paired P-cell stimulation (Fig. 1*c-f*). Each combination of P-cell inputs was associated with a unique pattern of motor neuron excitation and inhibition that accounted for one of the three forms of local bending.

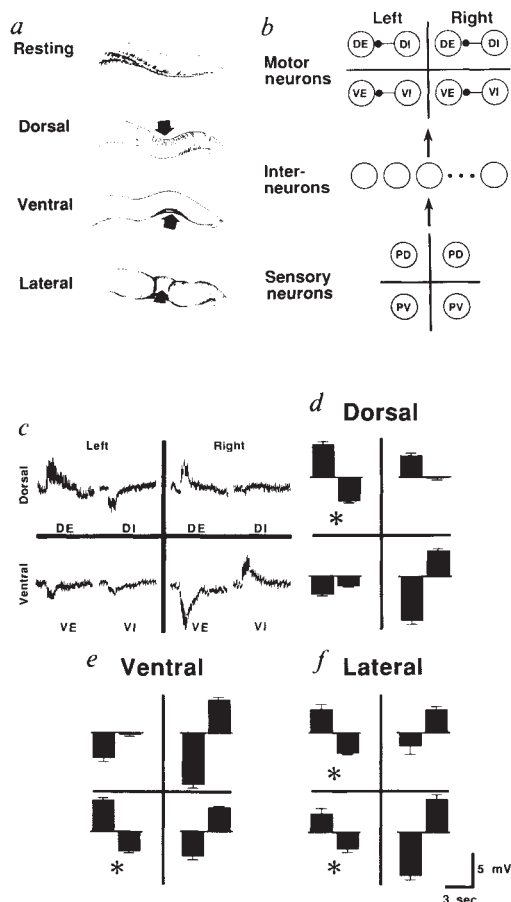


FIG. 1 Neural basis of local bending. *a*, Behaviour. Dorsal, ventral and lateral stimuli produce U-shaped local bends. *b*, Simplified neural circuit. The main input to the reflex is provided by the dorsal and ventral P cells (PD and PV)¹. Control of local bending movements is largely provided by motor neurons whose field of innervation is restricted to single left-right, dorsal-ventral quadrants of the body; dorsal and ventral quadrants are innervated by both excitatory (DE and VE) and inhibitory (DI and VI) motor neurons^{4,5}. Inhibitors strongly inhibit excitors of the same body quadrant, as the filled circles indicate¹¹. Weaker connections (not shown) exist from DIs to VIs (W. O. Friesen, personal communication), and also between homologous motor neurons (for example, left and right VEs)⁵. For simplicity, only a single tier of interneurons was assumed and the weaker connections between motor neurons were omitted. Connections between interneurons were not included as tests of a subset of the many possible connections between local bending interneurons revealed no functional connections (S.R.L.). *c-f*, Input-output function. *c*, Intracellular recordings from each type of neuron during stimulation of the left PD (10 Hz for 0.5 s). Within each quadrant input to the excitator is on the left, that to the inhibitor on the right. *d*, Average peak synaptic potential for the same motor neurons in response to left PD stimulation (same stimulus as *c*). *e*, Left PV stimulation. *f*, Simultaneous left PD and PV stimulation. Analogous data were obtained for simultaneous stimulation of both PDs, both PVs, and PV with the contralateral PD (not shown). These data were used to construct the training set for the model network—the list of associations between input and output patterns the model was trained to reproduce.

We searched isolated segmental ganglia for interneurons which are excited by a dorsal P cell and activate the two motor neurons exciting dorsal longitudinal muscles, thereby contributing to dorsal bends. In a survey of 73 ganglia, we found 9 morphologically distinct, repeatably identifiable interneurons with these connections (Fig. 2). Eight were bilaterally paired neurons and one was unpaired; each ganglion thus contains at least seventeen interneurons for dorsal bending. For the two interneurons for which connections to the remaining motor neurons could be tested, these connections too were largely consistent with the dorsal bending motor pattern (Fig. 1*d*).

Most dorsal-bending interneurons received three or four sensory inputs and all but one (cell 161) received excitatory input from ventral P cells. Thus, most of the dorsal-bending interneurons were active during lateral and ventral bending, when many of their motor neuron effects were inappropriate. The inappropriate effects of dorsal bending interneurons must

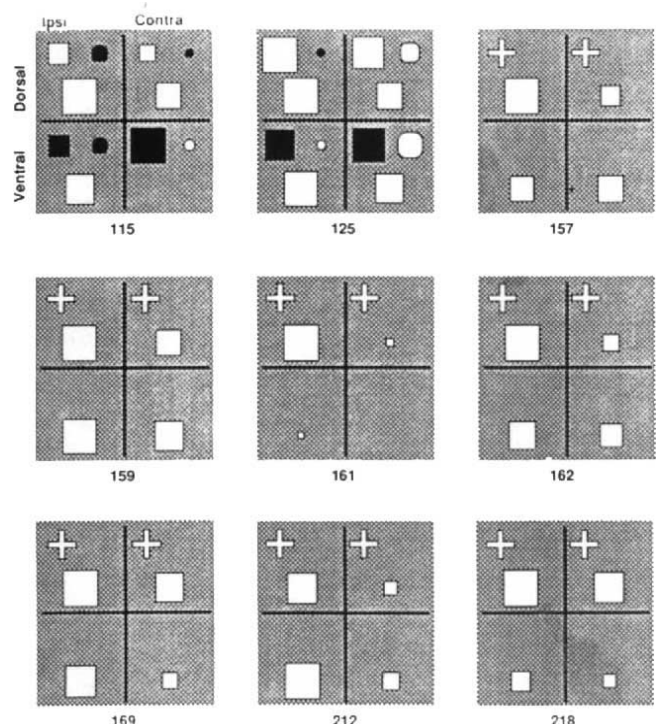


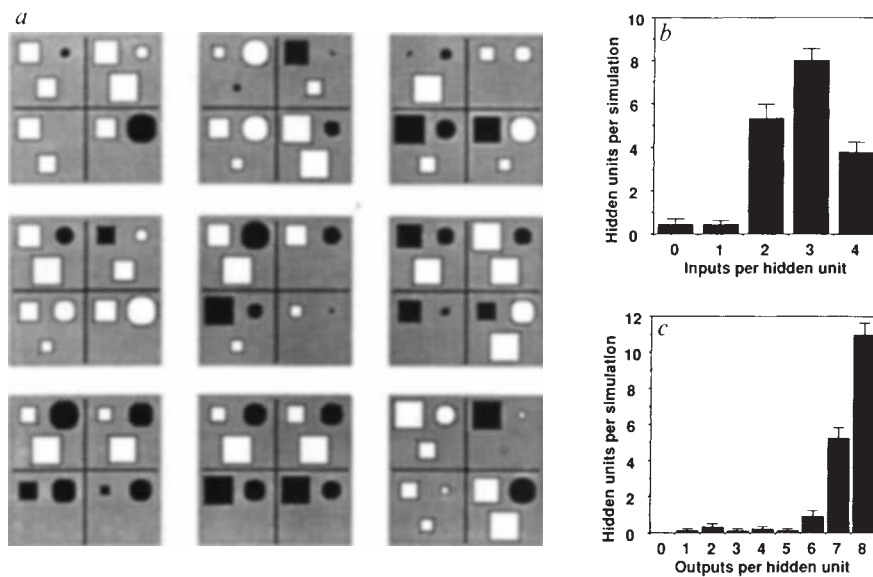
FIG. 2 Input and output connections of the nine identified local-bending interneurons, represented as 'cross diagrams'. Within each quadrant, the lower box represents the input connection from the P cell with its receptive field in the indicated body quadrant; the upper boxes represent output connections to the excitors (square boxes) and inhibitors (rounded boxes) that innervate the indicated body quadrant. Box area is proportional to synaptic strength of inputs and outputs for the indicated interneuron. Excitatory connections are white; inhibitory connections black. Pluses, excitatory connections of unknown weight determined by extracellular motor neuron recordings. Each ganglion contained one unpaired (cell 218) and eight bilaterally paired interneurons that are excited by PD input and that in turn excite DEs. With the exception of cell 115, which has been identified previously as an oscillator interneuron for swimming¹², none of these was previously identified. Hyperpolarization of individual interneurons during local bending generally produced a small decrement in response (S.R.L.). Input connection strengths were inferred from the heights of intracellular recordings of synaptic potential from the identified interneurons shown while each of four P cells was stimulated to produce identical impulse trains (10 Hz for 0.5 s). Output connection strengths were inferred from the heights of synaptic potentials recorded from motor neurons in response to current injection in an interneuron at physiological levels (0.5–2.0 nA for 3 s). Determination of output connections was limited for technical reasons to the two interneurons lying on the same surface of the ganglion as the motor neurons.

be offset by other interneurons. Local bending could therefore be the result of simultaneous activation of a population of interneurons with multiple sensory inputs and both appropriate and inappropriate effects on all eight motor neurons. It was not obvious, however, that such a population was sufficient, given the nonlinearities of neural elements and constraints imposed by connections known to exist in the network. The possibility remained that interneurons specific for each form of behaviour, or other as yet undiscovered types of interneurons, were required to produce the quantitative aspects of each output pattern.

FIG. 3 Model networks trained to produce local bending. *a*, Hidden units from one network after training. Connections of one member of each bilaterally symmetric pair shown. Symbols as in Fig. 2. *b* and *c*, Average numbers of hidden units from each of 18 different networks with a given number of significant input (*b*) or output (*c*) connections. Bars are standard error.

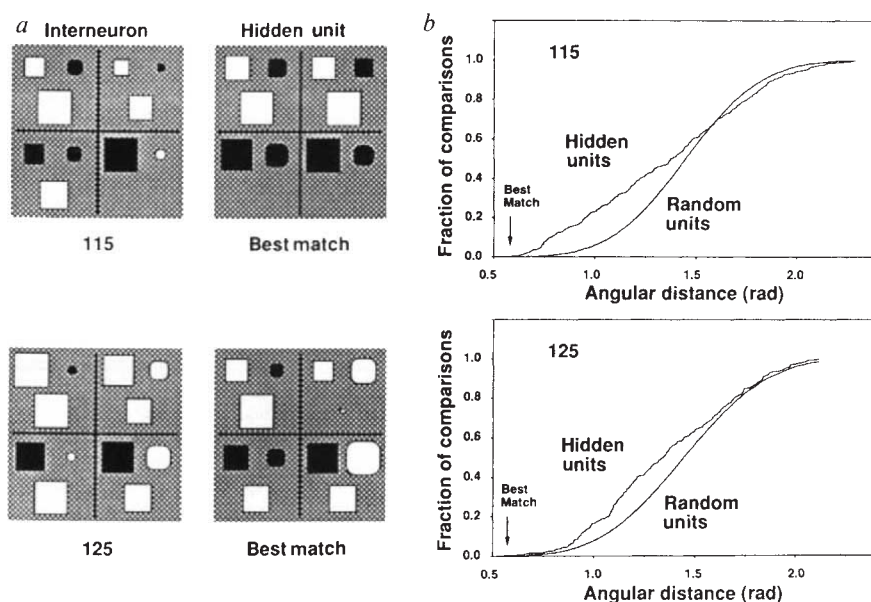
METHODS. Each network contained a single layer of 18 bilaterally paired hidden units, one pair for each type of interneuron. Training consisted of three steps. Step 1: input and output connections of the first nine hidden units were given small, randomly assigned weights. Input weights were positive, output weights were positive or negative. Connections of the other nine units were assigned weights making them mirror-images of the first set. Inhibitory connections between output units were assigned fixed negative weights. Step 2: an input pattern from the training set was presented and the hidden unit activation values calculated to determine the input to the output units. The activation of the inhibitory output units was then used to determine their input to the excitatory output units. Step 3: the net input values of the output units were subtracted from target values for that pattern (peak response of motor neurons in millivolts, Fig. 1*c-f*). This difference was used to determine the change in each input and output weight as required by the training algorithm² with the additional provision that mirror-image relations be preserved. Mirror-image relations were preserved by averaging the change in weight dictated

To address these issues, we used back-propagation to train a network of model neurons. The network had four input units representing the four P cells, and eight output units representing the eight motor neuron types. Between input and output units was a single layer of 18 hidden units representing the interneurons. Neurons were represented as simple processing units whose activation is a sigmoidal function of net synaptic input². Initially, synaptic connections, or weights, were small and randomly assigned; the task of the back-propagation algorithm was then to adjust the weights so that the network produced dorsal,



by the algorithm for each pair of related connections and changing each member of the pair by this value. Weights of connections from input to hidden units were not allowed to become negative. Steps 2 and 3 were repeated until the net input to the output units was within 5% of target for all 10 patterns in the training set. This required 10^4 – 10^5 presentations of the training set.

FIG. 4 Quantitative comparison of neurons and hidden units. *a*, Cells 115 and 125 with their closest matching hidden units. Neurons and hidden units were represented as 12-dimensional vectors with one dimension for each synaptic weight. The angle between the vectors of each neuron and hidden unit was determined. Best matches were the vector pairs with the smallest angle (cell 115, 0.547 rad; cell 125, 0.546 rad). *b*, Statistical significance of comparisons between neurons and hidden units. Hidden unit curves: x-values represent the set of angular distances for cell 115 or 125 compared to each of 324 hidden units; y-values show the fraction of all hidden units at an angular distance from the indicated neuron $\leq$ the x-value. Random unit curves: x-values are the same as above; y-values represent the fraction of randomly generated units at an angular distance $\leq$ the x-value. Random units ($n > 40,000$) were generated by selecting four input weights and eight output weights from pooled input and output weights of all hidden units. The random units thus had exactly the same distribution of weights as the hidden units. The random unit cumulative frequency distribution estimates the theoretical random probability of each neuron-hidden unit match. The standard error of the random unit curve is less than 2.5×10^{-3} . The probability of obtaining a match for cell 115 as good or better than the one shown in *a* was $3.4 \pm 0.92 \times 10^{-4}$; for cell 125 it was $3.8 \pm 0.97 \times 10^{-4}$. The shift of



the hidden unit cumulative frequency distributions toward better matches was highly significant (Kolmogorov-Smirnov test: cell 115, $D=0.19$, $n=324$, $P < 0.001$; cell 125, $D=0.17$, $N=324$, $P < 0.001$).

ventral and lateral local bending when the appropriate input units were activated. Back-propagation was solely to accomplish this task, not because of any presumed biological significance. The algorithm was constrained by three aspects of known connections: all connections from input to hidden units were excitatory (see Fig. 2); inhibitory connections between motor neurons in the same quadrant were included and assigned fixed, physiologically determined weights; and inputs and outputs of a given unit were the mirror image of its homologue to reflect the bilateral symmetry of the leech nervous system. No constraints were placed on the number of input and output connections.

Eighteen networks were trained; in each case, the algorithm found a different set of hidden units that accurately reproduced the local bending input-output function (Fig. 3a). Each of the resulting 324 (18 × 18) hidden units was unique. Many resembled identified dorsal local-bending interneurons in several respects: >95% received multiple sensory inputs and 88% had connections to seven or eight output units (Fig. 3b, c). To determine whether the similarity between hidden units and interneurons was attributable to their related functions, we compared cells 115 and 125, the two interneurons for which the relative strengths of all connections were known, to each of the 324 hidden units and also to 40,000 randomly generated, non-functional units. Neurons, as well as hidden and random units, were represented as 12-dimensional vectors, each dimension corresponding to a synaptic strength or weight. Similarity of neurons to hidden and random units was assessed by comparing the angular distance between vectors for cells 115 or 125 and each hidden or random unit. In the comparisons to random units, the likelihood of obtaining angular distances as small or smaller than those found in the best matches to hidden units (Fig. 4a) was in the order of 1 in 3,000. Also, comparison of the cumulative frequency of angular distances from cells 115 and 125 to hidden and random units (Fig. 4b) showed that there were proportionately more close hidden units than close random units. These comparisons show that the degree of similarity between real interneurons and hidden units was not accidental but directly attributable to the fact that model networks reproduced the local bending input-output function.

The similarity between model and actual interneurons demonstrates that a population of interneurons resembling the identified dorsal local-bending interneurons could mediate local bending in a distributed processing system without any additional interneuron types. Back-propagation is an essential tool in our demonstration because it enabled us to construct a quantitative model of the reflex from a partial set of data. In addition, our results quantitatively support previous observations that back-propagation naturally converges to a class of network solutions adopted by real nervous systems⁶⁻⁸. The absence of recurrent (feedback) connections in the local bending circuit makes it ideally suited to this type of analysis; however, the back-propagation algorithm has been adapted to the analysis of more complex, recurrent neural networks that occur in a wide range of nervous systems^{9,10}. □

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Division and differentiation of isolated CNS blast cells in microculture

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THE mechanism of transformation of the overtly similar cells of the neural plate into the numerous and diverse cell types of the mature vertebrate central nervous system (CNS)¹ can better be understood by studying the clonal development of isolated CNS precursor cells. Here I describe a culture system in which blast cells (cells capable of division) isolated from embryonic day 13.5-14.5 rat forebrain can divide and differentiate into a variety of clonal types. Most clones contain only neurons or glia; 22% contain both neurons and non-neuronal cells. For the division of blast cells, live conditioning cells need to be present indicating that environmental signals influence proliferation. Heterogeneous clones develop in homogeneous culture conditions, so factors intrinsic to the blast cells are probably important in determining the number and type of clonal progeny.

Dividing cells are present throughout the developing rat CNS, including in the septal region of the forebrain, on embryonic day 13.5-14.5 (E13.5-14.5) (refs 2 and 3). Septal tissue from the E13.5-14.5 rat brain was dissected and mechanically dissociated into a cell suspension. Single cells were then isolated from the suspension and transferred to the bases of Terasaki wells by micromanipulation so that there was one septal cell per well, as described in the legend to Fig. 1. Each well contains culture medium conditioned by a primary bulk culture of cells from the striatal region of the E13.5-14.5 rat brain growing on the walls of the wells. I chose striatum initially as a source of conditioning cells because it is an abundant developing tissue close to the septal region. The wells are observed by phase microscopy at 1-2 hours after plating; most of them (88%) contain a single septal cell. The fate of these single cells is followed by daily photography.

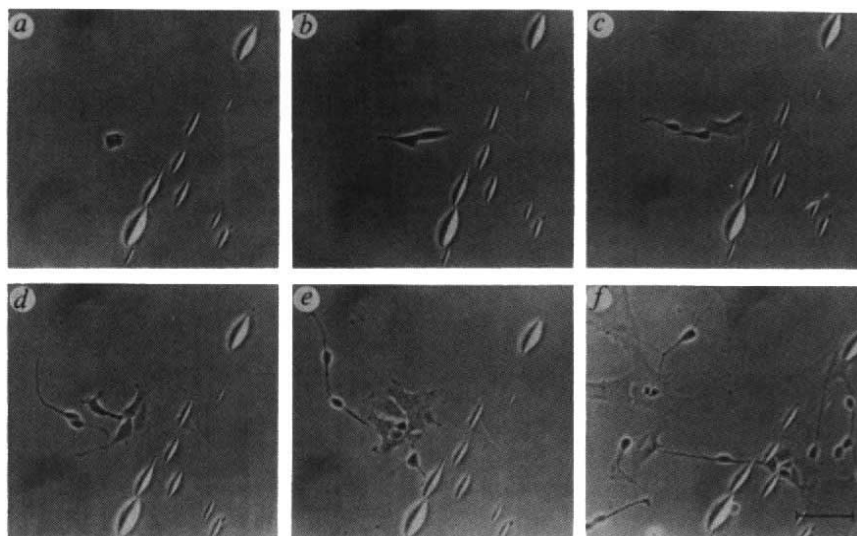
More than 90% of the septal cells survive the first 24 h of culture and, on average, 19% divide during this time. Dividing cells are defined as blast cells: 58% of the blast cells divide once and the remaining 42% go on to give larger clones. In control experiments over 200 wells with feeder cells on the walls but with no septal cells were observed for 7-14 days; none contained single CNS cells or groups of cells in the central region of the well base used to study developing clones. The presence of live conditioning cells is critical for blast-cell division: when single septal cells are plated into wells containing conditioned medium transferred from striatal cell cultures rather than live conditioning cells, only 1-2% of the septal cells divide, and none of these divide more than once. The viability of single cells is comparable whether or not conditioning cells are present: ~70% of the wells contain live septal cells after 3 days *in vitro*. These results suggest that the conditioning cells are releasing a short-lived, soluble factor important for blast-cell division. Conditioning cells derived from E14.5 cortex and E13.5-14.5 septum also support the development of septal clones, so the soluble activity is probably not specific to the striatal region. Figure 1 shows the initial development of one of the larger septal clones grown in the presence of striatal conditioning cells, which contained 34 cells after 10 days *in vitro*.

The composition of developing clones is summarized in Table 1. Neurons are identified by their rounded soma and long, fine processes and by positive staining with an antibody to neurofila-

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FIG. 1 The development of a large, mixed clone from a septal blast cell. *a*, Single cell after 2 h *in vitro*. *b*, Two daughter cells after one day *in vitro* (d.i.v.). *c*, Four cells after 2 d.i.v. *d*, Six cells after 3 d.i.v. *e*, 12 cells after 4 d.i.v. *f*, Portion of culture after 8 d.i.v., by which time the neurons and non-neuronal cells are clearly distinguishable morphologically. The scratches in the plastic visible on the right side of all the pictures were created by the glass electrode when the original blast cell was plated. They serve to identify the field (see also Fig. 3). Scale bar, 50 μ m.

METHODS. Freshly dissected tissue from the septal region of the E13.5–14.5 rat brain was dissociated by gentle, mechanical trituration in culture medium N5 (ref. 14) supplemented either with 5% neuronal survival factor (ref. 15) (a neurotrophic, acid-stable fraction of horse serum of relative molecular mass 55,000) or with 10% fetal calf serum (FCS) (Gemini Bioproducts). A dilute cell suspension was transferred to a bacteriological Petri dish containing N5 plus 10% FCS. Large cells, constituting less than 10% of the cells in a typical suspension, were micromanipulated using a hand-pulled glass microelectrode and transferred to the bases of wells of a Terasaki plate, one cell per well. Large cells were selected as a means of enriching for blast cells as it was found on plating that most small cells in the suspension were post-mitotic neurons. Each well had a pre-established culture of E13.5–14.5 striatal cells growing on its walls but not on its base. These wall cultures were established by inoculating each poly-L-lysine coated well with 10 μ l of a suspension of striatal cells in N5 plus 10% FCS ($0.15\text{--}0.5 \times 10^6$ cells per ml.) while holding the Terasaki plate in an inverted position. The plates were incubated in this position for 6 days and then



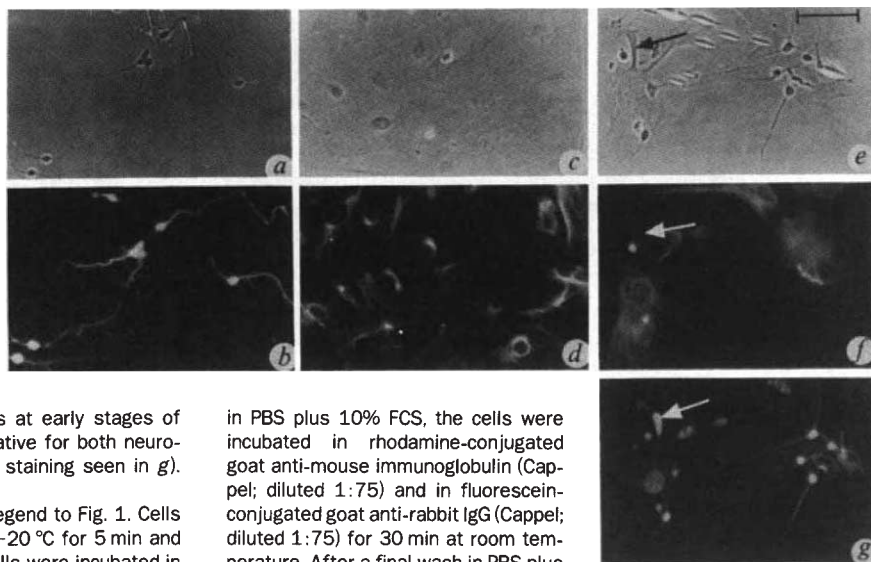
righted just before changing the medium 1–3 times to wash out any cells remaining in suspension. The wells then contained 10 μ l of N5 plus 10% FCS, which was allowed to condition for 3–7 days. Twenty per cent concanavalin-A-activated spleen cell-conditioned medium (T-cell monoclonal, Collaborative Research) was added to the wells immediately before plating the single cells. Clones were fed by replacing 2 μ l medium with fresh culture medium every 3–5 days. Cells were cultured in 6% CO₂ at 100% humidity and 35 °C.

ment protein. Non-neuronal cells are identified by morphology (usually fibroblast-like) and negative staining with neuronal markers, and characterized further with serum against glial fibrillary acidic protein (GFAP). Not all clones were stained immunohistochemically, but there was >90% correlation between the morphological and immunohistochemical identification of neuronal and non-neuronal cells. As shown in Table 1, most clones (70%) give rise to neurons alone and 67% of these contain two neurons. The highest number of neurons seen

in a purely neuronal clone was eight; an example of such a clone is shown in Fig. 2*a, b*. Eight per cent of the blast cells give rise to only non-neuronal cells and 75% of these clones contain GFAP-positive astrocytes⁴ (Fig. 2*c, d*). The remaining 22% of the clones contain both neurons and non-neuronal cells, the number of neurons varying from 1–17 and the number of non-neuronal cells from 1–60 (Table 1). Fifty five per cent of the mixed clones contain GFAP-positive astrocytes as well as neurons. The clone in Fig. 1 is an example of this type: after

FIG. 2 Immunohistochemical identification of cells in clones derived from septal blast cells. *a* and *b*, Clone of 8 cells after 8 d.i.v., shown in *a* to have the morphology of neurons by phase-contrast microscopy and in *b* to have positively stained processes after staining with anti-neurofilament antibody. (Note that the antibody cross-reacts with a component of the cell nuclei.) *c* and *d*, Clone of GFAP-positive astrocytes stained after 9 d.i.v.: *c*, phase-contrast micrograph and *d*, fluorescence micrograph after staining for GFAP. *e–g*, Portion of the clone shown in Fig. 1 after 10 d.i.v.: *e*, phase contrast micrograph; *f* and *g*, the same field after double-labelling with anti-GFAP serum and anti-neurofilament-antibody respectively. Both neurofilament-positive neurons and GFAP-positive astrocytes are present in this clone. Arrows in *e–g* indicate a cell with characteristics of blast cells at early stages of clonal development (small, flattened appearance, negative for both neurofilament and GFAP and has the typical bright nuclear staining seen in *g*). Scale bar, 75 μ m.

METHODS. Clones were cultured as described in the legend to Fig. 1. Cells were fixed in 95% ethanol, 5% glacial acetic acid at –20 °C for 5 min and washed with phosphate-buffered saline (PBS). Fixed cells were incubated in PBS plus 10% FCS for 1 h at room temperature (22 °C) and then incubated for 30 min at room temperature in mouse monoclonal anti-neurofilament antibody 3F11 (Chemicon; culture supernatant diluted 1:6 in PBS plus 10% FCS) and in rabbit anti-GFAP (ref. 16) serum diluted 1:600. After washing



in PBS plus 10% FCS, the cells were incubated in rhodamine-conjugated goat anti-mouse immunoglobulin (Cappel; diluted 1:75) and in fluorescein-conjugated goat anti-rabbit IgG (Cappel; diluted 1:75) for 30 min at room temperature. After a final wash in PBS plus 10% FCS, wells were filled with 25 mg ml^{–1} sodium iodide in 95% glycerol, 5% PBS, pH 8.6, to prevent fading of the fluorescence¹⁷. Cells were viewed in a Nikon diavert microscope and photographed on Kodak T-max 400 and P3200 film.

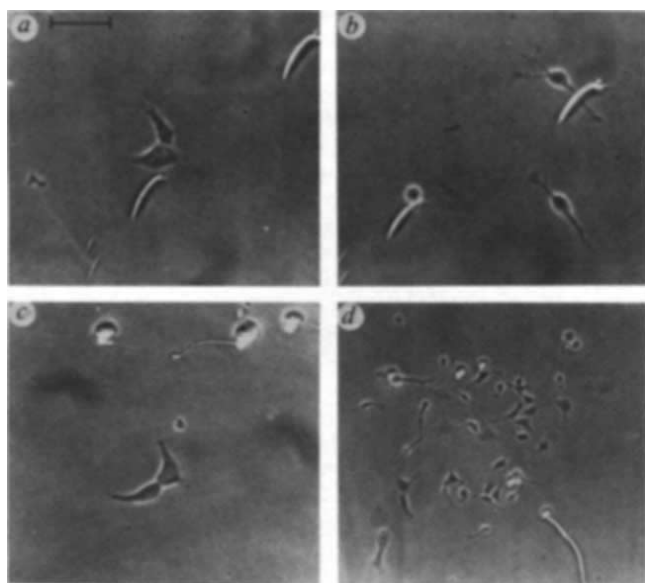


FIG. 3 Two clones that were initially similar in appearance but later diverged to yield different progeny. *a* and *c*, Phase-contrast micrographs of two apparently similar clones each at the two-cell stage after 1 d.i.v. *b* and *d*, Subsequent development of the clones in *a* and *c* respectively: *b*, clone of 3 neuron-like cells (at this stage of development, immature neurons often round up and have either short or no processes) taken after 4 d.i.v. and *d*, a large mixed clone taken after 6 d.i.v. Scale bar *a*–*c*, 45 μ m; *d*, 85 μ m.

10 days *in vitro* there are 12 cells with neuron-like morphology (ten of which neurofilament-positive), 20 GFAP-positive astrocytes and two cells that do not stain for either marker and whose morphology is similar to the blast cell that originally gave rise to the clone. The immunochemical staining of this clone is shown in Fig. 2*e*–*g*. The remaining 45% of mixed clones contain neurons and GFAP-negative, non-neuronal cells. Most GFAP-negative non-neuronal cells are morphologically similar to GFAP-positive cells. None of the progeny in any of the clones has oligodendrocyte morphology; most clones were fixed and stained by 7 days *in vitro*, so this lack of oligodendrocytes is consistent with a later appearance of these cells in developing rat brain⁵.

The septal blast cell has a characteristic flattened shape *in*

vitro, with prominent, phase-dark inclusions often visible in the cytoplasm (Fig. 1). Overtly similar blast cells consistently give rise to clones of different composition. For example, Fig. 3*a* and *c* shows two clones that were morphologically similar at the two-cell stage, but one differentiated into three neuron-like cells (Fig. 3*b*) and the other yielded a mixed clone of 42 cells (Fig. 3*d*). Although some variations in blast cell morphology were noted, I found no consistent relationship between blast-cell morphology and subsequent clonal development.

The difference in clone types indicates that there are three basic types of blast cell in the E13.5–14.5 septal region. The first undergoes a limited number of divisions and differentiates into a single-cell type, as evidenced by the purely neuronal or glial clones. The second also undergoes a limited number of divisions, but differentiates into both neurons and glial cells, which is consistent with the existence of a common precursor for these two cell types in other areas of the CNS^{6–11}. The third type of blast cell gives rise to large, mixed clones which continue to divide and contain a few cells with characteristics of the initial septal blast cell, even after long periods of culture. These may be multipotential stem cells which can generate several cell types and can renew themselves¹².

These data describe the development of E13.5–14.5 septal blast cells, isolated as single cells *in vitro*, to give rise to clones containing neurons or glia, or neurons and glia. The progeny types and the time course of their appearance *in vitro* is consistent with normal septal development^{2,3}, so these culture conditions might provide a good system for studying the generation of CNS cells. An important benefit of the culturing of isolated cells is that their environment can be controlled more precisely than in mass cultures or *in vivo*. The single-cell culture system has been used previously to distinguish the environmental and cell-intrinsic factors that influence the development of glial blast cells from the rat optic nerve¹³. My experiments separate the contributions of these factors in the development of neurons and glia in the forebrain. Live conditioning cells are necessary for blast-cell division, suggesting that an environmental signal influences septal-cell proliferation. Analysis of retinal development has led to the conclusion that the differentiation of multipotential retinal precursor cells is also largely under environmental control^{6–8}. In the experiments described here, the environment around single septal cells was standardized, but the cells gave rise to clones that vary considerably in size and in the number and type of progeny they contain, implying that these cells contain intrinsic factors specifying different fates. Preliminary results indicate that single cells from embryonic cerebral cortex and hippocampus probably also divide and differentiate in similar culture conditions. Thus the single-cell culture system may be useful for studying the development of blast cells from various regions of the CNS. □

TABLE 1 Composition of 130 clones from embryonic E13.5–14.5 rat septal cells in microculture

Neuronal or non-neuronal clones			Mixed clones of neuronal and non-neuronal cells		
Number of neuronal cells in clone	Number of non-neuronal cells in clone	Frequency of observation	Number of neuronal cells in clone	Number of non-neuronal cells in clone	Frequency of observation
2	0	61	1–2	1–8	13
3–4	0	23	2	60	1
5–8	0	7	3–4	2	2
0	2–4	3	4	20	1
0	5–8	2	5–8	1–4	6
0	9–16	3	10–13	22–26	2
0	17–32	3	16–17	2–7	2
			16	37	1
Total clones		102			28

Culture techniques are described in the legend to Fig. 1. Neuronal and non-neuronal cells are defined morphologically and immunohistochemically. Rare cells with the characteristics of early septal blast cells (Fig. 2) that are seen in some mixed clones are included in the non-neuronal population.

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Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation

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RECENT studies in invertebrates indicate that a rapid genomic response to neuronal stimulation has a critical role in long-term changes in synaptic efficacy¹. Because several of the genes (immediately early genes; IEGs) that respond rapidly to growth factor stimulation of vertebrate cells *in vitro*²⁻⁷ are also activated by neuronal stimulation *in vivo*⁸⁻¹³, attention has focused on the possibility that they play a part in synaptic plasticity in vertebrate nervous systems. Four IEGs thought to encode transcription factors, *zif/268*⁵ (also termed *Egr-1*¹⁴, *NGFI-A*¹⁵, *Krox 24*¹⁶), *c-fos*¹⁷, *c-jun*¹⁸, and *jun-B*⁷ are rapidly induced in the brain by seizure activity^{8,11,13}, and we have now studied the induction of these genes in a well-characterized model of synaptic plasticity in the vertebrate brain—long-term potentiation (LTP) of the perforant path-granule cell (pp-gc) synapse *in vivo*¹⁹. We found that high-frequency (but not low-frequency) stimulation of the pp-gc synapse markedly increases *zif/268* messenger RNA (mRNA) levels in the ipsilateral granule cell neurons; mRNA of *c-fos*, *c-jun* and *jun-B* is less consistently increased. The stimulus frequency and intensity required to increase *zif/268* mRNA levels are similar to those required to induce LTP, which is also seen only ipsilaterally, and both responses are blocked by NMDA-receptor antagonists as well as by convergent synaptic inhibitory inputs already known to block LTP²⁰. Accordingly, *zif/268* mRNA levels and LTP seem to be regulated by similar synaptic mechanisms.

Continuous low-frequency stimulation of the perforant path (pp) does not produce LTP or alter IEG mRNA levels in the dentate gyrus (Fig. 1). By contrast, brief high frequency (HF) stimulation of the pp capable of inducing LTP^{21,22} markedly increases *zif/268* mRNA levels in granule cell neurons of the ipsilateral dentate gyrus 30 or 60 min after the stimulus (Fig. 1; Table 1). Whereas highly reproducible increases in *zif/268* mRNA were seen following HF stimuli of the pp (12 out of 13 animals), increases in *c-fos*, *c-jun* and *jun-B* mRNA were observed in only four, six and seven of the same thirteen animals, respectively. This relatively selective increase in *zif/268* mRNA following high-frequency stimulation of the pp differs sharply from the coordinate mRNA increases of the same IEGs induced by pharmacological¹³ or maximal electroconvulsive seizures (MECS) (manuscript in preparation) and is consistent with a previous report that the *c-fos* protein is not induced by HF stimulation of the pp¹². In subsequent studies, we therefore focused on characterizing the *zif/268* mRNA response.

Seizures induce increases in *zif/268* mRNA throughout the dentate gyri bilaterally. Increases following high-frequency stimulation of the pp however, are strictly unilateral and appear more intense in the dorsal than the ventral hippocampus. This discrete distribution of enhanced *zif/268* mRNA levels supports the premise that it reflects pp fibre activation, because most pp fibres terminate ipsilaterally²³ and the stimulating electrode is positioned to maximize synaptic potentials in the dorsal hippocampus.

A characteristic feature of IEGs is that their mRNA levels increase rapidly and transiently after specific stimuli^{3,24}. After

high-frequency stimulation of the pp, *zif/268* mRNA levels displayed such a pattern, increasing within 15 min ($n=2$), attaining highest levels between 30 and 60 min, and returning to basal levels by 3 h ($n=6$).

Induction of LTP by high-frequency synaptic stimulation at many synapses, including the pp-gc synapse²⁵, involves stimulation of NMDA (*N*-methyl-D-aspartate) receptors²⁶. Therefore, we determined whether increases in *zif/268* mRNA induced by high-frequency stimulation are also mediated by NMDA receptor activation. Intraperitoneal (i.p.) administration of (+)MK-801 (1 mg kg⁻¹), a non-competitive NMDA receptor antagonist²⁷ that blocks LTP²⁸, blocked the increase in *zif/268* mRNA in the dentate gyrus normally induced by high-frequency stimulation of the pp, whereas (−)MK-801 (1 mg kg⁻¹, i.p.), which

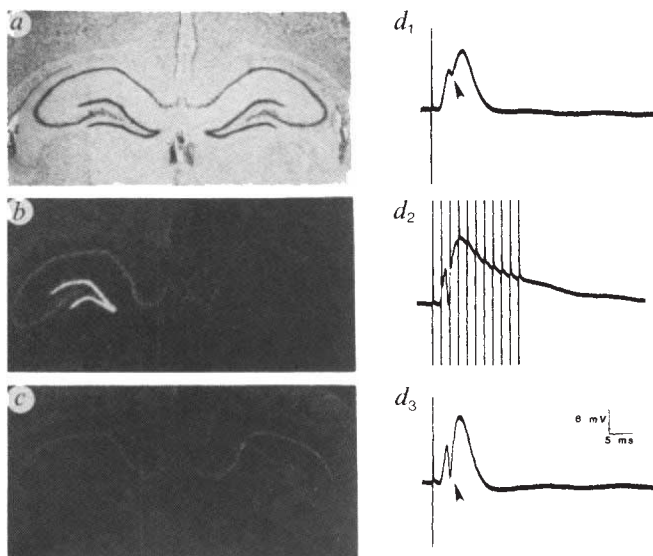


FIG. 1 High-frequency stimulation of the perforant path induces increases in *zif/268* mRNA in granule cell neurons. *a*, Rat dorsal hippocampus ($\times 5$). Tissue section stained with Cresyl violet shows the molecular and granule cell layers of the dentate gyrus. Perforant path afferents synapse on granule cell dendrites in the molecular layer. *b*, *c*, Autoradiographs of *in situ* hybridizations with ³⁵S-labelled *zif/268* riboprobe to rat dorsal hippocampus following *in vivo* orthodromic stimulation of granule cells via pp afferents. *b*, 1 h after a high-frequency pp stimulus composed of 12 20-ms bursts of monophasic pulses (50 μ s) at 500 Hz, repeated at 0.1 Hz. The intensities of the individual pulses were sufficient to elicit a maximal PS response at 0.1 Hz (60 V). In 12 of 13 preparations (data pooled from animals killed 30 or 60 min after stimulation), hybridization was unilaterally increased in the dentate gyrus ipsilateral to the stimulating electrode (left side). Levels of *zif/268* mRNA induced by the high-frequency pp stimulus are similar to those induced in the dentate gyrus by pharmacological seizures¹³ or MECS (Fig. 2). Low frequency stimulation. Synaptic responses to single stimuli were monitored every 10 s throughout a 1-h recording period before death. Hybridization remained at basal levels in all of nine preparations. *d*, Recordings from the dentate gyrus of the animal illustrated in *b*. *d*₁, Perforant path stimulation evokes a broad upgoing e.p.s.p. and superimposed PS (arrowhead). *d*₂, Synaptic potential induced by a high-frequency stimulus. *d*₃, Synaptic potential recorded 1 h after the stimulus train. The stimulus intensity is the same as in *d*₁. Note potentiation of the PS amplitude (arrowhead).

METHODS. Standard *in vivo* recording techniques^{21,22} were used with little modification. Adult male Sprague-Dawley rats (175–225 g) were anaesthetized with chloral hydrate (400 mg kg⁻¹, i.p.) and electrodes were placed in the dorsal dentate gyrus and ipsilateral pp at the angular bundle. Stimuli were delivered by a digital timer driving an adjustable voltage stimulus isolation unit. Electrical potentials were amplified and recorded with a digital oscilloscope. Stimulating and recording electrodes were in the left hemisphere and synaptic responses were monitored every 10 s throughout the recording period in each case (25 V, 50- μ s test pulse). Because seizures¹³ and kindling stimuli¹¹ activate IEGs, spontaneous and evoked electrical activity was monitored and no after-discharges or spontaneous bursts were observed. Animals were killed by decapitation and experimental brains were routinely co-mounted with brains from non-operated animals and from animals 30 min after MECS (UGO Basile ECT unit 7801; 85 mA, 100 Hz, 500 μ s pulses, 1 s duration) to provide stimulated and naive controls in the same histological section. *In situ* hybridization was performed as previously described¹³. To ensure specificity of *in situ* hybridization, stringent conditions were maintained during probe hybridization, and wash steps were based on empirically determined melt conditions. No hybridization of sense-strand probes was detected. RNase A pretreatment of tissue sections blocked hybridization of nick-translated cDNA probes. ³⁵S-riboprobe complementary to the 3' non-coding region of *zif/268* gave results identical to those obtained using a full length (3.2-kilobase) antisense probe.

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is relatively inactive at the NMDA receptor²⁷, did not (Fig. 2). Additionally, the competitive antagonist CGS-19755²⁹ (10 mg kg⁻¹, i.p.), which blocks neuronal responses mediated by NMDA receptors *in vivo*³⁰, blocked the effect of high-frequency stimulation of the pp on *zif/268* expression. Neither (+)MK-801 (10 mg kg⁻¹) nor CGS-19755 (15 mg kg⁻¹) blocked increases in *zif/268* mRNA induced by MECS (Fig. 2). These results indicate that NMDA receptor activation is necessary for high-frequency stimulation of the pp to induce increases in *zif/268* mRNA, and emphasize differences between IEG responses to such stimuli and to seizures.

LTP of the pp-granule cell synapse can be blocked by a crossed inhibitory input through activation of the contralateral dentate hilus²⁰. Stimulation of this pathway immediately before, but not after, stimulation of the pp-granule cell synapse blocks LTP, presumably by reducing activation of postsynaptic NMDA receptors. We therefore assessed whether convergent inhibitory inputs also alter mRNA levels of *zif/268*. Results indicate that mRNA levels of *zif/268* and LTP are either induced or blocked in parallel (Fig. 3). This demonstrates that specific synaptic pathways control *zif/268* mRNA levels, presumably through modulation of events mediated by NMDA receptors.

Although NMDA-receptor activation seems to be critical in triggering both LTP and increases in *zif/268* mRNA, these responses may require quantitatively different degrees of NMDA-receptor activation. To induce LTP reliably the intensity of a train of high-frequency stimuli must exceed a critical threshold^{21,22}. This threshold is thought to produce the minimum activation of the NMDA receptor necessary to induce LTP²⁶ and is identified as the intensity required for a single pulse to elicit a minimum population spike (PS). Therefore, we administered high-frequency stimuli to the pp composed of pulses either at or below this electrophysiological threshold. We

TABLE 1 Effect of stimulus type on *zif/268* mRNA and LTP

Stimulus type	Number of animals	Number of animals showing:		
		Increased <i>zif/268</i> mRNA	LTP	LTP and increased <i>zif/268</i> mRNA
Low frequency (0.1 Hz)	9	0	0	0
Subthreshold (500 Hz)	6	1	2	0
Threshold (500 Hz)	13	6	8	5
Maximal (500 Hz)	13	12	11	10

Relationship of stimulus frequency and intensity to increases in *zif/268* mRNA. The low frequency group is described in Fig. 1. HF (frequency parameters, see Fig. 1) stimulus trains of three different intensities were defined by the amplitude of the PS. For subthreshold stimulation, the voltage was adjusted to just below that at which individual pulses at 0.1 Hz elicit an excitatory postsynaptic potential (e.p.s.p.) without a PS. For the threshold-intensity group, the stimulation voltage was adjusted to elicit <25% of the maximal PS. The maximal PS group is described in Fig. 1. Animals were killed 30–60 min after the stimulation. Just before death, presence of LTP was determined by comparing the PS amplitude and e.p.s.p. slope at submaximal stimulus levels. Animals that displayed >50% potentiation of submaximal PS amplitude or >30% increase in e.p.s.p. slope were scored positive for LTP. To score *in situ* hybridization results, optical densities (OD) of autoradiograms were measured over the granule cell layers (Loats Image Analysis System). An increase in OD of $\geq 30\%$ between the stimulated dentate and the contralateral side in at least two sections was scored as positive for an increase in *zif/268* mRNA. In the low frequency group, differences in OD never exceeded 5%. Statistical analysis of these data demonstrated that more intense stimuli induce *zif/268* mRNA more frequently ($P=0.0018$; logistic regression analysis), and indicate that threshold stimuli are sufficient to induce *zif/268* mRNA ($P<0.05$; χ^2 comparison of threshold stimulus with subthreshold and low frequency stimulus groups).

found that increased expression of *zif/268* is induced more frequently with increased stimulus intensity and that the minimum stimulus intensity required to induce LTP is similar to that required to produce an increase in *zif/268* mRNA (Table 1).

FIG. 2 NMDA antagonists block *zif/268* mRNA increases induced by high-frequency stimuli; *zif/268* *in situ* autoradiograms. *a*, 30 min after a high-frequency pp stimulus as in Fig. 1. Animals were pretreated 1 h before the pp stimulus with either (+)MK-801 (upper panel) or (-)MK-801 (lower panel) (1 mg kg⁻¹, i.p.). (+)MK-801 blocked the increase in *zif/268* probe hybridization in five of six preparations whereas (-)MK-801 failed to block the effect in each of two preparations. Low-frequency synaptic potentials appeared normal in these preparations. *b*, 30 min after MECS. Animals were pretreated for 1 h with either (+)MK-801 (10 mg kg⁻¹) (upper panel) or an identical volume of solvent (ethanol) (lower panel). (+)MK-801 failed to block MECS induced increases in *zif/268* mRNA ($n=3$).

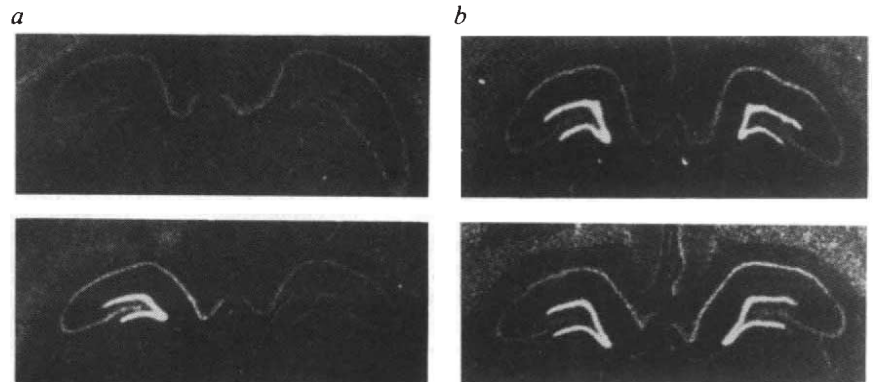
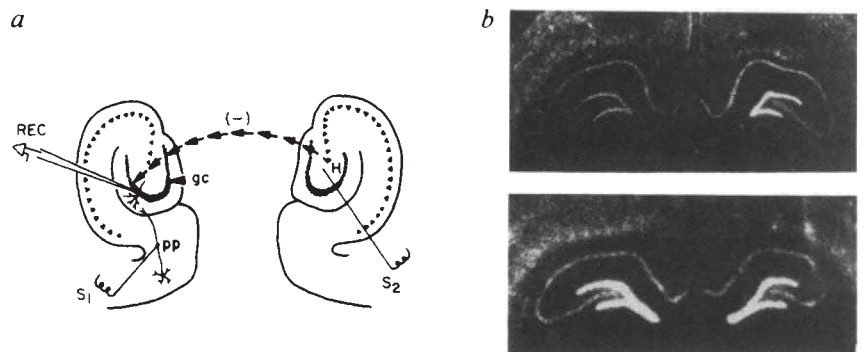


FIG. 3 Inhibitory projections from the contralateral dentate hilus block high-frequency pp stimulus-induced increases in *zif/268* mRNA in the dentate gyrus. *a*, Electrode positions and crossed inhibitory input. Standard stimulating (S_1) and recording (REC) electrodes were positioned as described in Fig. 1. The intensity of the S_1 stimulus was adjusted to evoke a maximum PS response at 0.1 Hz (60 V). A second bipolar stimulating electrode (S_2) was placed in the contralateral dentate hilus and the intensity adjusted so that an S_2 stimulus delivered 10 ms before S_1 blocked the orthodromically evoked PS (60–80 V). At these defined intensities, high-frequency stimulus trains (as described in Fig. 1*a*) were delivered to both S_1 and S_2 . LTP is blocked if S_2 immediately precedes S_1 but not if their order is reversed²⁰. *b*, *zif/268* *in situ* autoradiograms. In three of four preparations in which S_2 preceded S_1 , *zif/268* mRNA in the orthodromically stimulated dentate gyrus remained at basal levels (top panel; left dentate gyrus). By contrast, if S_1 preceded S_2 , typical increases in *zif/268* mRNA were induced by the HF pp stimulus (bottom panel; left dentate gyrus, $n=2$).



Stimulation of the dentate hilus directly with the S_2 electrode enhanced *zif/268* mRNA levels in the ipsilateral dentate gyrus in all preparations (top and bottom panels; right dentate gyri). However, stimulation of S_2 alone did not change *zif/268* mRNA levels in the contralateral (left) dentate gyrus ($n=2$).

In most animals, LTP and an increase in *zif/268* mRNA coincide; in a few, however, we noted a dissociation between these responses. Although both responses seem to require similar degrees of NMDA-receptor activation, this dissociation may represent differential activation of other steps in the cascades leading to the electrophysiological and mRNA responses. Alternatively, it may reflect technical limitations in detecting localized or small increases in *zif/268* mRNA.

In summary, these results indicate that the amount of *zif/268* mRNA is rapidly increased by synaptic mechanisms that involve activation of NMDA receptors. This increase shares several features with LTP, a well-characterized NMDA-dependent phenomenon, including induction by high but not low-frequency stimuli, blockade by NMDA-receptor antagonists and by convergent inhibitory inputs, and similar stimulus-intensity thresholds. Because *zif/268* encodes a presumed transcription regulatory factor, it could be important in coordinating changes in gene expression that maintain synaptic plasticity. Studies of the transcriptional regulation of *zif/268* could provide insight into the transduction processes leading from NMDA-receptor activation to gene expression. □

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High-frequency nanometre-scale vibration in 'quiescent' flagellar axonemes

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THE movement of cilia and flagella is based on the interaction between dynein arms and microtubules coupled with ATP hydrolysis. Although it is established that dynein arms cause adjacent microtubules to slide, little is known about the elementary process underlying the force production. To look more closely at the mechano-chemical conversion mechanism, we recently developed an optical method for measuring a nanometre-scale displacement with a time-resolution better than 1 ms. We now report the detection of high frequency (~300 Hz) vibration of sub-nanometre amplitude in non-beating flagellar axonemes. This vibration could reflect the movement of individual activated dynein arms.

The ATPase activities of dynein can be activated by the presence of microtubules^{1–3}. Thus dynein has higher ATPase activities when contained within an axoneme than when isolated. Such ATPase activation is a prerequisite for a dynein-microtubule system to work efficiently⁴. Curiously, however, activation takes place even in axonemes that are rendered non-motile. We suspected that an uncoordinated force would be produced in such quiescent axonemes with activated ATPase activities, and therefore looked for structural fluctuations in the axoneme.

Flagellar axonemes of sea urchin sperm had their membranes removed and were fragmented and placed between a glass slide and a coverslip. Polystyrene microbeads were attached to the axonemes (Fig. 1), and the specimen was perfused with a reactivation solution. These axonemes did not beat, despite the presence of ATP, because they were detached from heads. The

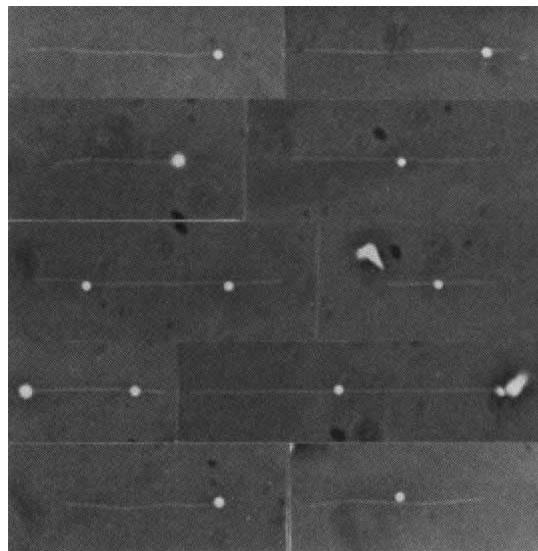


FIG. 1 Phase-contrast images of polystyrene microbeads attached to fragmented flagellar axonemes. Scale bar, 10 μ m.

METHODS. Sea urchin (*Hemicentrotus pulcherrimus*) spermatozoa were demembrated at 4 °C with a solution containing 0.04% (w/v) Triton X-100, 10 mM Tris-HCl (pH 8.0), 150 mM KCl, 2 mM MgSO₄, 1 mM EDTA and 1 mM dithiothreitol (DTT). After being kept in this solution for 30–60 s, the axonemes were transferred to a standard buffer solution containing 20 mM Tris-HCl (pH 8.0), 200 mM potassium acetate, 2 mM MgSO₄, 1 mM EGTA, 0.5 mM EDTA and 1 mM DTT, and fragmented with a Teflon homogenizer. The fragmented axonemes were introduced between a glass slide and a coverslip held 0.8 mm apart with a pair of coverslip strips. After standing for 2–3 min, axonemes that did not attach to the glass surface were washed out by perfusion with the standard buffer solution. Polystyrene microbeads (Polybead-Carboxylate Monodisperse Microbeads, Polyscience Co.) of 0.9 μ m diameter were attached to the axonemes by perfusing the sample with a 0.025% (w/v) suspension in the standard buffer. ATPase activity of the axoneme preparation was measured in the presence of 1 mM ATP after sperm heads were removed by centrifugation¹. The liberated phosphate and the protein concentrations were determined by the methods of Anner and Moosmayer²³ and of Lowry *et al.*²⁴, respectively.

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movement of the axoneme-bound microbead was then analysed with a device⁵ that can detect displacements of less than 1 nm.

The frequency spectra of movement from most microbeads displayed a monotonic amplitude decrease with increasing frequency. Such a spectrum should represent thermally-induced random movements, because it was observed even in the absence of ATP (Fig. 2*f*). In 10–40% of the total axoneme-bound beads, however, we observed a striking oscillation, revealed by a sharp peak in the fast Fourier transform (FFT) spectra, of a remarkably high frequency: ~300 Hz at an ATP concentration of 1 mM (Fig. 2*d*) of amplitude <1 nm. The direction of vibration was variable from one bead to another, although in many cases the amplitude of vibration was largest in the direction of the axoneme axis. This vibration lasted stably for more than 30 min;

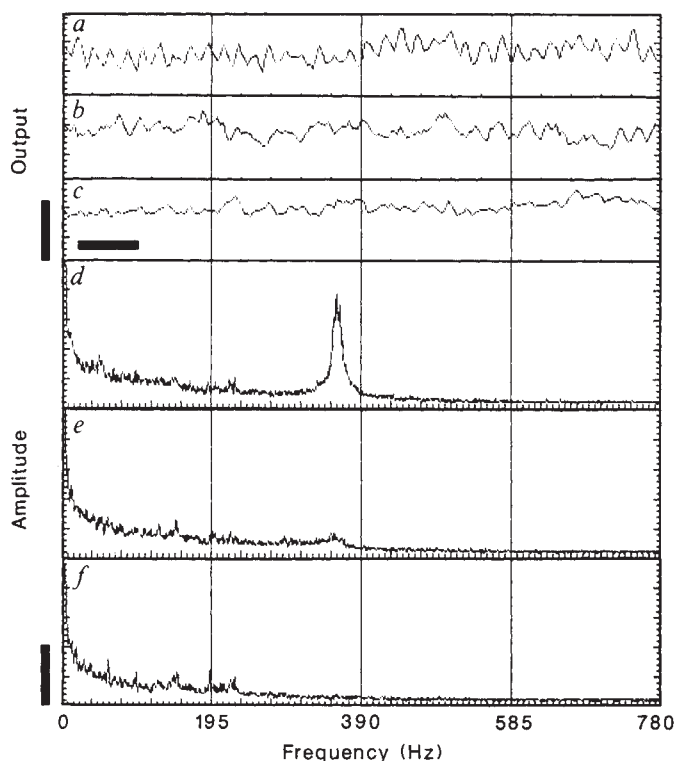


FIG. 2 ATP-dependent vibration of a microbead attached to an axoneme fragment. *a*, Output from the optical detector showing the vibration in the presence of 1 mM ATP (494 μ M Mg-ATP). Vertical bar, 1 V (corresponding to 9 nm); horizontal bar, 10 ms. *b*, *c*, Output for the same bead as in *a* in the presence of 1 mM ATP and 0.5 μ M orthovanadate (*b*), or in the absence of ATP (*c*). *d*, *e*, *f*, FFT patterns of the outputs shown in *a*, *b* and *c*, respectively. Vertical bar, 20 mV (corresponds to a displacement of 0.18 nm).

METHODS. Fragmented axonemes with beads attached were perfused with reactivating solutions (standard buffer solution containing 0.025–1 mM ATP (Boehringer)). Nanometre-scale displacement of the bead was monitored at various ATP concentrations, as described previously⁵. This method is based on improvements of a technique previously used to monitor the motion of glass needles with submicrometre precision^{25–28}. Here a microscope image of each bead was divided into two parts, the image intensities of which were compared by using photodiodes and a differential amplifier. The intensity difference was found to be sensitive to a displacement as small as 1 nm and proportional to the one-dimensional displacement up to ~1 μ m; the output voltage was calibrated with a known amount of displacement produced by a piezoelectric actuator. Movements of microbeads were usually analysed with the optical sensor set parallel to the axonemal axis so that axial movements were measured; when information about motion in other directions was needed, the microscope stage was rotated. The output of the apparatus (about 90 mV per nm displacement) was analysed with a computer (Model 9801RX, NEC) equipped with an a.c.–d.c. converter and a program for FFT analysis (Canopus Electronics, Kobe, Japan). Sampling rate of the a.c.–d.c. converter was 2 kHz. FFT calculation was with 2,048 data points sampled for 1 s, and 20–40 transforms were averaged to obtain a final pattern. A Hanning's window function was used.

it was so stable that we could measure frequencies with the same bead under different conditions by repeated perfusions (Fig. 3). We often observed that the FFT spectrum from a single microbead had two (but never three) discrete peaks at close but different frequencies, for example, at 280 and 300 Hz. The significance of this phenomenon remains to be determined.

The vibration frequency depended on the MgATP concentration in a Michaelis–Menten manner (Fig. 4). From double reciprocal plots, the apparent Michaelis constant (K_m) and the maximal vibration frequency were determined to be 94 μ M and 340 Hz. The apparent K_m value is similar to those obtained from the dependence on MgATP of the flagellar beat frequency^{6–8} and of the sliding velocity of microtubules^{9,10}, suggesting that dynein ATPase activated by microtubules is responsible for this movement. This conclusion is supported by the observation that the high-frequency vibration was reversibly blocked by orthovanadate (0.5 μ M) (Fig. 2*e*), a potent inhibitor of dynein ATPase^{11–13}. Orthovanadate seemed to inhibit the vibration by reducing its amplitude but not its frequency.

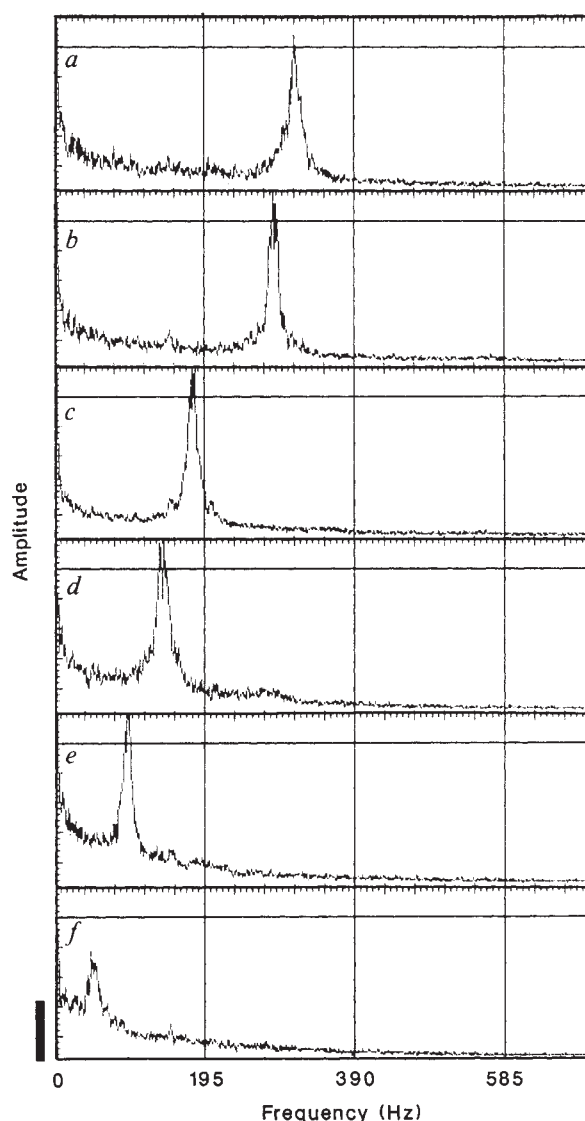


FIG. 3 FFT spectra of amplitude from the output obtained at different ATP concentrations. Vertical bar, 20 mV (corresponds to a displacement of 0.25 nm). The MgATP concentration and peak frequency in each case were: 494 μ M, 315 Hz (*a*); 274 μ M, 287 Hz (*b*); 117 μ M, 180 Hz (*c*); 59.4 μ M, 139 Hz (*d*); 30 μ M, 96 Hz (*e*); and 15 μ M, 51 Hz (*f*). In this particular example, a single microbead was used for repeated measurements for 15 min, during which the vibration frequency did not change as long as the MgATP concentration was constant. MgATP concentration was calculated as described²⁹.

The vibration was also observed with normal-length axonemes with sperm heads attached; in this case, microbeads were not needed for the measurement, because the bright-head image could be directly used to analyse the movement. Gibbons and Gibbons reported¹⁴ that demembrated sperm becomes quiescent in a reactivation solution with an elevated concentration of calcium ions. They suggested that this quiescent state represents an active state in which many dynein crossbridges remain potentiated to produce sliding force. We found that the heads of such quiescent sperm also vibrated at a high frequency: 290 ± 17 Hz at an ATP concentration of 1 mM (average of nine measurements). This raises the possibility that the axonemes are vibrating at a high frequency while dynein arms are generating sliding force.

We were unable to observe the high-frequency vibration in axonemes from which a bundle of outer-doublet microtubules had been extruded after perfusion with elastase and ATP^{15,16}. This indicates that either an intact axonemal structure or the presence of inter-doublet links is necessary for the vibration, as it has been suggested that elastase (a protease) preferentially disrupts the inter-doublet links¹⁵. The intact axonemal structure or inter-doublet links may be required to provide antagonistic forces needed for the vibration¹⁷; such antagonistic forces may be maintained only in cylindrically closed systems, because dynein arms exert unidirectional force on adjacent microtubules¹⁸.

The axonemal ATP hydrolysis rate under the same conditions as used above was $\sim 2 \times 10^7$ ATP molecules per mg axoneme per min, which, if we assume that the outer and inner arms are identical, corresponds to an average turnover rate of ~ 20 ATP molecules per s for a single dynein arm (ATP concentration of 1 mM). This result is in agreement with previous data^{1,19}. However, only some of the dynein arms might have undergone ATPase activation in the above experiments. In fact, dynein arms crosslinked to microtubules have recently been reported to have an ATP turnover rate as high as 150 molecules per s (T. Shimizu, S. P. Marchese-Ragona and K. A. Johnson, personal communication). Hence, the maximal rate of ATP hydrolysis by a single dynein can be close to, or even identical with, the vibration frequency of ~ 300 Hz. Thus the high-frequency vibration may be tightly coupled with the ATPase cycle. The observation that orthovanadate reduced the vibration amplitude but not its frequency also supports the idea that the vibration frequency reflects the turnover of individual activated dynein, because orthovanadate is known to reduce the number of active dynein arms by bringing them to a kinetic dead end²⁰. We cannot, however, exclude the possibilities that the vibration is caused by cooperative movements of many arms, or by multiple steps in a crossbridge cycle.

We have demonstrated here the feasibility of measuring a

sub-nanometre-scale movement (see also ref. 21) and the presence of a high-frequency vibration in a dynein-microtubule system. Vibration at such a high frequency has not been observed in other eukaryotic motility systems except that of insect flight muscle²². Because nanometre-scale molecular movement may occur in various cellular mechanisms, it will be interesting to apply our method of study to other biological systems. □

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Homology of 54K protein of signal-recognition particle, docking protein and two *E. coli* proteins with putative GTP-binding domains

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MOST proteins exported from mammalian cells contain a signal sequence which mediates targeting to and insertion into the membrane of the endoplasmic reticulum (ER)^{1,2}. Involved in this process are the signal-recognition particle (SRP) and docking protein (DP), the receptor for SRP in the ER membrane¹. SRP interacts with the signal sequence on nascent polypeptide chains and retards their further elongation¹, which resumes only after interaction of the arrested ribosomal complex with the docking protein^{3,4}. SRP is a ribonucleoprotein particle comprising a 7S RNA and six polypeptides with relative molecular masses (M_r) of 9,000 (9K), 14K, 19K, 54K, 68K and 72K (ref. 1). The 9K and 14K proteins are essential for elongation arrest and the 68K–72K heterodimer is required for docking to the ER membrane⁵. The 54K protein binds to the signal sequence when it emerges from the ribosome^{6,7}. Docking protein consists of two polypeptides, a 72K α -subunit

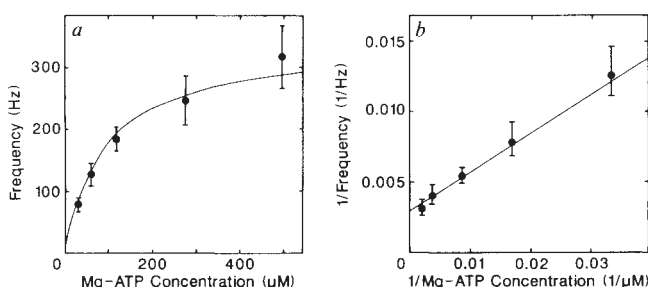


FIG. 4 Dependence of the high-frequency vibration on MgATP concentration. *a*, Mean frequencies and standard deviations (vertical bars) obtained from data with different axonemes. Temperature, 21 ± 1 °C. For each data point, 11–79 samples were measured. *b*, Double reciprocal plot of the data in *a*. This plot yields an apparent K_m of 94 μ M MgATP and a maximal frequency of 340 Hz.

(DP α) and a 30K β -subunit (DP β)⁸. No components structurally homologous to SRP and docking protein have yet been found in yeast or *Escherichia coli*. To understand the molecular nature of the interaction between the signal sequence and its receptor(s) we have characterized a complementary DNA coding for the 54K protein of SRP. Significant sequence homology was found to part of DP α and two *E. coli* proteins of unknown function. The homologous region includes a putative GTP-binding domain.

An expression cDNA library⁹ from cytoplasmic poly(A)⁺ RNA of MDCK (Madin Darby canine kidney) cells was screened with a monoclonal antibody against the 54K subunit of SRP (SRP54)¹⁰. One positive clone was identified. The cDNA insert in this clone was 1.4 kilobases (kb) in length. Using northern blot analysis on messenger RNA of MDCK cells, we estimated the size of the full-length mRNA for SRP54 to be ~2.5 kb (Fig. 1a). After rescreeing the library, a 2.4 kb cDNA clone was selected and analysed by *in vitro* transcription and translation (Fig. 1b, c). The translation product is a 54K protein which co-migrates with SRP54 isolated from canine pancreas and can be immunoprecipitated with an anti-SRP54 antibody (Fig. 1b).

The SRP54 cDNA was sequenced. It is 2,321 base pairs (bp) in length and has a single large open reading frame encoding 504 amino acids (Fig. 2); the calculated M_r of the encoded protein is 55.7K. Amino-acid sequences obtained from the N terminus and from tryptic peptides of SRP54 match the deduced amino-acid sequence (Fig. 2). The ATG initiation codon is preceded by 376 nucleotides which contain two ATG codons and two in-frame stop codons (Fig. 2, boxed). The first ATG is directly followed by a stop codon; the second ATG is followed by an open reading frame of 16 codons. Translation in eukaryotes usually starts at the first ATG¹¹. It has been shown, however, that ATG/stop codon combinations preceding the initiation site can reduce translational efficiency¹¹. We therefore deleted most of the 5' flanking region, including the first two ATG codons from pSRP54 (Fig. 1c). Messenger RNA derived from the resulting plasmid (pSRP54-1, Fig. 1c) is translated more efficiently than that from pSRP54 (data not shown).

SRP can be reconstituted from isolated 7S RNA and SRP proteins^{12,13}. We tested binding of the cDNA encoded 54K protein to an SRP19/7S RNA complex formed *in vitro*. Association of SRP54 with the complex was observed only in the presence of both 7S RNA and the 19K subunit of SRP (SRP19) (Fig. 3). Thus the components synthesized *in vitro* can interact with each other specifically and form an SRP subparticle.

The SRP54 amino-acid sequence was compared with the Swissprot, NBRF, EMBL and Brookhaven data libraries using the FASTA program of Pearson and Lipman¹⁴. We found a striking similarity to three proteins: the DP α ; a 48K *E. coli* protein with unknown function¹⁵; and the *E. coli* FTS Y protein which is encoded by a gene of a cell division operon¹⁶. Regions of homology are outlined in Fig. 4a and an alignment of the sequences is shown in Fig. 4b. Identical amino acids are shaded in grey. The region between amino acids 100 and 300 of SRP54 is most conserved among all four proteins (Fig. 4). Comparison of the consensus sequence derived from the alignment with the data libraries revealed a weak similarity of the domain corresponding to amino acids 166–227 of SRP54 with amino acids 312–376 of the 70K heat-shock proteins (HSP70). HSP70 proteins have an affinity for hydrophobic surfaces in unfolded proteins¹⁷, which might resemble the interaction of SRP54 with the signal sequence.

Over its entire length SRP54 is very similar to a 48K *E. coli* protein¹⁵. This protein has been identified as an open reading frame upstream of the *trmD* operon at 56 min on the *E. coli* chromosome¹⁵. The N-terminal 60% of SRP54 is homologous to the C-terminal half of DP α and of the FTS Y protein. Proteolytic cleavage experiments suggested that the N-terminal 151 amino acids of DP α are required for association with the ER membrane and that mixed-charge amino-acid clusters surround-

ing the elastase cleavage site (see arrow Fig. 4a) are supposedly involved in interaction with SRP¹⁸. The FTS Y protein is encoded by a gene in the filamentation temperature sensitive (*fts*) operon at 76 min on the *E. coli* genetic map. Mutations in this operon affect essential cellular functions including heat-shock response and cell division¹⁶. The function of the FTS Y protein is not known.

Components involved in protein secretion in *E. coli* have been characterized using either genetic or biochemical techniques². Several groups have described partial purification of cytoplasmic factors and oligomeric protein complexes which stimulate protein translocation across membranes in *E. coli* and in yeast systems *in vitro*. None of these proteins seems to be identical with either the 48 K or the FTS Y protein.

The region of strongest similarity between the four proteins (amino acids 100–300 of SRP54) contains three highly conserved sequence elements indicative of guanine-nucleotide-binding sites (Fig. 5). By X-ray crystallography of the elongation factor EF-Tu (ref. 19) and the Ha-ras product p21 (ref. 20) it has been shown that these sites are in contact with the GTP and correspond to loops at the ends of adjacent parallel β -strands^{19,20}.

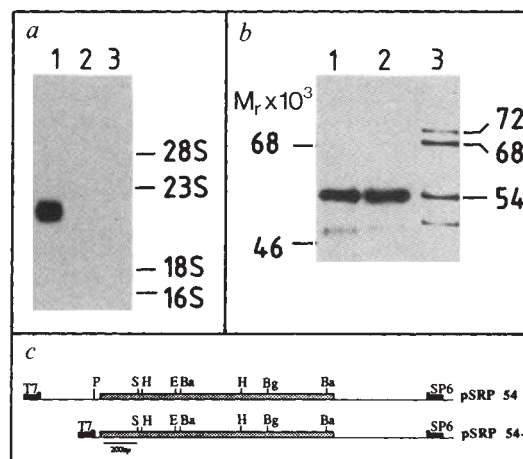


FIG. 1 a, Northern blot analysis of poly(A)⁺ RNA from MDCK cells (lane 1). Control, total RNA from canine pancreas (lane 2) and *E. coli* (lane 3), using pSRP54Tex cDNA as probe. b, Translation products of mRNA obtained by *in vitro* transcription from pSRP54. Lane 1, total translation; lane 2, immunoprecipitation with the monoclonal anti-SRP54 antibody¹⁰; lane 3, 72K, 68K and 54K SRP proteins. c, Schematic representation of SRP54 specific cDNA clones. T7, SP6, promoter of bacteriophage T7 and SP6 respectively; restriction-enzyme cleavage sites: P, *Pst*I; S, *Sph*I; H, *Hind*III; E, *Eco*RI; Ba, *Bam*HI; Bg, *Bgl*II. The black boxes indicate multiple restriction sites and the shaded areas SRP54 coding regions.

METHODS. A cDNA library was prepared from cytoplasmic poly(A)⁺ RNA, isolated from MDCK cells essentially as described by Haymerle *et al.*⁹ in the bacterial expression vector pTEx (Herz *et al.*, in preparation). 2×10^5 colonies were screened using a monoclonal antibody against SRP54¹⁰. One positive clone was found (pSRP54Tex). Cytoplasmic poly(A)⁺ RNA of MDCK cells and total cytoplasmic RNA of canine pancreas and *E. coli* were separated on a 1% agarose-formaldehyde gel¹³ and transferred to gene screen plus membrane (NEN). A fragment comprising the whole pSRP54Tex cDNA (1.4kb) insert was labelled by random priming and hybridized according to ref. 26. Filters were washed at 65 °C in 1 mM EDTA, 40 mM NaHPO₄ (pH 7.2), 1% SDS. Ribosomal RNA from dog pancreas and *E. coli* were used to calculate the approximate size of SRP54 mRNA. The MDCK cDNA library was rescreeed for full-length clones with pSRP54Tex. Twenty-nine additional positive clones were found and their insert sizes tested. For *in vitro* expression, one of the longest cDNA clones was subcloned into the *Sma* site of pGem-2 (Promega) (c, pSRP54). Plasmids were then transcribed with either T7 or SP6 polymerase²⁷ and the transcripts translated in the wheat germ cell-free system. Protein products were characterized by SDS-PAGE and fluorography (b). SRP proteins (b, lane 3) were visualized by silver staining²⁸. To generate pSRP54-1, the 5' proximal region of pSRP54 was removed with *Pst*I (see Fig. 3).

reaction partners²². GTP binding and hydrolysis are thought to produce two different protein conformations with different affinities for an effector molecule. GTP-binding proteins include elongation and initiation factors, and also proteins which mediate the vectorial transport of secretory vesicles. In the latter process GTP is thought to mediate vesicle targeting and vesicle fusion²².

-361
TGGCAATCGTCGGTCC

-340 -320 -300 -280 -260 -241
TCCAGCTGGTGGGAGTTGGCGTCGCTGTGCTGGGCGCTGGAGCCCTAGTTTGTATAGTTTGGGAAGTCGGGCTTGGAGTCGCACCTGTCTGTCTGACCCCTGCTTCTCTGGTTCCCTT

-220 -200 -180 -160 -140 -121
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-100 -80 -60 -40 -20 -1
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MetLeuPheProValLeuHisThrAlaAlaAspPheLeuValIleThrThr

20 40 60 80 100 120
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MetValLeuAlaAspLeuGlyArgLysIleThrSerAlaLeuArgSerLeuSerAsnAlaThrIleIleAsnGluGluValLeuAsnAlaMetLeuLysGluValCysThrAlaLeuLeu

140 160 180 200 220 240
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GluAlaAspValAsnIleLysLeuValLysGlnLeuArgGluAsnValLysSerAlaIleAspLeuGluGluMetAlaSerGlyLeuAsnLysArgLysMetIleGlnHisAlaValPhe

260 280 300 320 340 360
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LysGluLeuValLysLeuValAspProGlyValLysAlaTrpThrProThrLysGlyLysGlnAsnValIleMetPheValGlyLeuGlnGlySerGlyLysThrThrThrCysSerLys

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500 520 540 560 580 600
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GlySerTyrThrGluMetAspProValIleIleAlaSerGluGlyValGluLysPheLysAsnGluAsnPheGluIleIleIleValAspThrSerGlyArgHisLysGlnGluAspSer

620 640 660 680 700 720
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LeuPheGluGluMetLeuGlnValAlaAsnAlaIleGlnProAspAsnIleValTyrValMetAspAlaSerIleGlyGlnAlaCysGluAlaGlnAlaLysAlaPheLysAspLysVal

740 760 780 800 820 840
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AspValAlaSerValIleValThrLysLeuAspGlyHisAlaLysGlyGlyGlyAlaLeuSerAlaValAlaAlaThrLysSerProIleIlePheIleGlyThrGlyGluHisIleAsp

860 880 900 920 940 960
GATTTTGAACCTTTCAAACACAGCCTTTTATCAGCAGCTTCTTGGTATGGGTGACATCGAAGGACTGATAGATAAAGTCAATGAGTTGAAGTTGGATGACAATGAAGCACTTATACAG
AspPheGluProPheLysThrGlnProPheIleSerLysLeuLeuGlyMetGlyAspIleGluGlyLeuIleAspLysValAsnGluLeuLysLeuAspAspAsnGluAlaLeuIleGlu

980 1000 1020 1040 1060 1080
AAGTTAAAAACATGGTCAGTTTACGTTGCGAGACATGTATAACAATTTCAAAATATCATGAAGATGGGCCCTTCAGTCAGATCTTGGGTATGATCCCTGGTGTGTTGGTACAGATTTTATG
LysLeuLysHisGlyGlnPheThrLeuArgAspMetTyrGluGlnPheGlnAsnIleMetLysMetGlyProPheSerGlnIleLeuGlyMetIleProGlyPheGlyThrAspPheMet

1100 1120 1140 1160 1180 1200
AGCAAAGGAAATGAACAAGAGTCAATGGCAAGGCTAAAAAGTTAATGACGATAATGGATAGTATGAATGATCAAGAAGCTTGACAGTACTGATGGTGCCAGGTTTTTATAGTAAGCAACCA
SerLysGlyAsnGluGlnGluSerMetAlaArgLeuLysLysLeuMetThrIleMetAsnAspGlnGluLeuAspSerLysThrAspGlyAlaLysValPheSerLysGlnPro

1220 1240 1260 1280 1300 1320
GGAAGAATCCAGAGAGTAGCAAGAGGATCAGGTGTGTCAACAAGAGATGTTCAAGAACTTTTGACACAAATATACCAAAATTTGCACAGATGGTAAAAAAGATGGGAGGTATCAAAGGACTT
GlyArgIleGlnArgValAlaArgGlySerGlyValSerThrArgAspValGlnGluLeuLeuThrGlnTyrThrLysPheAlaGlnMetValLysLysMetGlyGlyIleLysGlyLeu

1340 1360 1380 1400 1420 1440
TTCAAAGGTGGTGACATGCTAAGAATGTGAGCCAGTCACAGATGGCAAACTGAATCAACAAATGGCCAAAATGATGGATCCAAAGAGTTCTTCATCATGGTGGTATGGCAGGACTT
PheLysGlyGlyAspMetSerLysAsnValSerGlnSerGlnMetAlaLysLeuAsnGlnGlnMetAlaLysMetMetAspProArgValLeuHisHisMetGlyGlyMetAlaGlyLeu

1460 1480 1500 1520 1540 1560
CAGTCCATGATCAGGCAGTTTCAACAGGGTGTGCTGGCAATATGAAAGGCATGATGGGATTCAATAATATGTAAGAAGATGCCTTAATTTAAACTGACTCAGTTGATTACATTATTTG
GlnSerMetMetArgGlnPheGlnGlnGlyAlaAlaGlyAsnMetLysGlyMetMetGlyPheAsnAsnMet

1580 1600 1620 1640 1660 1680
CTGAGACCTCAAGAGTTTCCCTCCTTTTTTGGCAACAGGAAAAAAAAGATTTTTCTTGCTGTCTTGCCCTTTTTCTTCTCTCTCATCTTCTTCTCTCTCTACTTCTCTCTCTCTCT

1700 1720 1740 1760 1780 1800
TTGATATAAGGGAGGAATATATAGTTTTTGTGGAAATCATTATATTTTGCCTTAGATTTCTTTTCTTCTAGTTTCACCATCATAACTACTTCTTAAGTTAAACAAATCATGATGTAAAAAT

1820 1840 1860 1880 1900 1920
CTAGTACTTAGAAGTTTCTAATTTGTCTCAAGGCCAAGCATTACATTTGTAACAGCTCCTGATCAGTAGTTATATGATGTCTCAATAAAAGTGCTGTAAATAAACTTCAAACCTCGTTAT

1940
AAAAAAAAAAAAAAAAAAAAAAAAAAAA

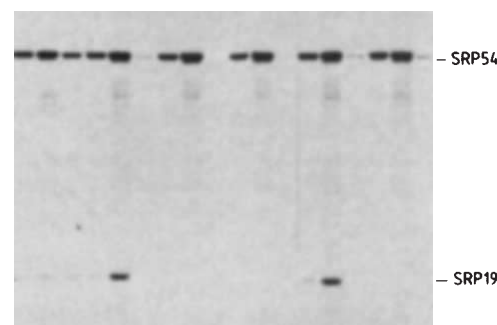
FIG. 2. Sequence of the SRP54 cDNA and the deduced amino-acid sequence of SRP54. The initiating methionine was identified by comparison with the protein sequence derived from the N-terminus of SRP54. Sequences obtained by sequencing tryptic peptides of SRP54 were found to be identical with the amino-acid sequence deduced from pSRP54. These sequences are underlined. The positions of two ATG and in-frame stop codons in the 5'untranslated region of pSRP54 are indicated by boxes. Polyadenylation signals (AATAAA) in the 3'untranslated region are underlined.

indicated in Fig. 1c) were subcloned into M13 vectors²⁹. Subclones were sequenced by the dideoxy-chain-termination method using Sequenase (USB) and gaps or ambiguities were resolved by using internal oligodeoxynucleotide primers. The N-terminal 8 amino acids of SRP54 were determined by the method of Matsudaira³⁰ after separation of SRP proteins by SDS-PAGE and blotting onto polyvinylidene-difluoride membranes. Internal amino-acid sequences were obtained after tryptic digestion of SRP54 protein (blotted onto nitrocellulose)³¹ followed by selection of peptides by HPLC.

FIG. 3 Binding of *in vitro* synthesized SRP54 to SRP19-7S RNA. The binding assays contained SRP 7S RNA (7S RNA), transfer RNA (tRNA), SRP54 (54) and SRP19 (19) in the combinations indicated on top of the figure. Total translations (T), complexes bound to DEAE Sepharose (B) and unbound (U) material were characterized by SDS PAGE and fluorography.

METHODS. Formation of an RNP particle was tested by a modification of a previously published method¹³. *In vitro* transcripts from pSRP19¹³ and from pSRP54-1 (Fig. 1c) were translated for 30 min at 25 °C in a wheat germ cell free system. EDTA was added to 5 mM final concentration, to release nascent chains from ribosomes and tRNA, and incubation was continued for 15 min. The translation reactions were then adjusted to 5mM magnesium acetate and 500 mM potassium acetate. SRP54 translation reaction (5 µl) SRP19 translation reaction and (10 µl) and 7S RNA or tRNA (1 µg) were mixed in appropriate combinations and incubated at 25 °C for 30 min. The volume was adjusted to 200 µl with 500 mM potassium acetate, 5 mM magnesium acetate, 50 mM Tris, pH 7.4, wash buffer. One-fifth of the reaction volume was removed and trichloroacetic acid (TCA) precipitated to monitor for total protein synthesis (T). DEAE-Sepharose CL-6B (Pharmacia) (30 µl) equilibrated in wash buffer was added to the remainder and incubated at 4 °C for 15 min. After centrifugation the unbound supernatant fraction (U) was TCA precipitated. The DEAE-Sepharose beads were washed twice with wash buffer and DEAE-bound material (B) released at 4 °C with 200 µl the same buffer

54	54	54	54	54	54
19	19	-	-	19	-
7S RNA	-	7S RNA	-	tRNA	tRNA
T U B	T U B	T U B	T U B	T U B	T U B



adjusted to 2 M KCl. Eluted protein was TCA precipitated and all samples analysed on 10–15% Laemmli-type SDS-polyacrylamide gels³² and subjected to fluorography.



FIG. 4 Alignment of protein sequences homologous between SRP54, an *E. coli* 48K protein, DP α and the *E. coli* FTS Y protein using the multiple sequence alignment algorithm of Vingron and Argos³³. The dark grey areas in *a* show the region of highest similarity between the four proteins. The light shaded area indicates a segment with weaker but still significant similarity. A region homologous between SRP54 and *E. coli* 48K only is

indicated by vertical hatching. Diagonal hatching at the N-terminus of hDP α indicates the domain with which this protein interacts with the DP β subunit in the ER membrane^{8,23}. The arrow shows the elastase cleavage site¹⁸. Sequences aligned for homology are shown in *b*^{15,16,34}. Identical amino acids are indicated by shading.

Consensus	G x x x x G K T/S	D x x G	N K x D
SRP54	108 G L Q G S G K T	190 D T S G	248 T K L D
<i>E. coli</i> 48K	107 G L Q G A G K T	190 D T A G	248 T K V D
hDP	425 G V N G V G K S	520 D T A G	588 T K F D
FTS Y	301 G V N G V G K T	382 D T A G	446 T K L D
N-RAS, human	10 G A G G V G K S	57 D T A G	116 N K C D
EF-Tu, <i>E. coli</i>	18 G H V D H G K T	80 D C P G	135 N K C D

FIG. 5 The three consensus sequence elements for a GTP-binding domain³⁵ are present in SRP54, *E. coli* 48K protein, hDP α and FTS Y. N-Ras and EF-Tu GTP-binding domains are shown for comparison³⁵. Numbers in front of the sequence motifs refer to amino-acid positions in the respective protein.

to and insertion into the membrane of the ER. This process could be envisaged as a sequential interaction of the signal sequence with several receptor molecules. SRP54 has been shown to interact with the signal sequence. The site of interaction has not yet been determined. Binding sites of ligand (effector) molecules in Ras proteins and in EF-Tu are thought to comprise a region between the first and the second GTP consensus site^{19,20}. The corresponding site in SRP54 might interact with the signal sequence. It is possible that the signal sequence, upon interaction of SRP with the docking protein, is translocated from the GTP-binding domain of SRP54 to the homologous domain of DP α or, with lower efficiency, is directly inserted into the ER membrane after release from SRP²³.

Prokaryotic and eukaryotic signal sequences are very similar²⁴. The structural similarity of the signals would suggest that their receptors share structural features as well. This hypothesis is supported by data from experiments which show the functional interaction of bacterial proteins with the eukaryotic secretion machinery²⁵. The striking similarity between the SRP54 and the *E. coli* 48K protein over nearly their entire length might point to a related function. DP α and the *E. coli* FTS Y protein lack the C-terminal domain that is homologous between SRP54 and the *E. coli* 48K protein. They both have non-homologous extensions at the N terminus. The presence of the *fts y* gene in a locus affecting cell division might point to a membrane related function of the FTS Y protein. Protein export from *E. coli* is thought to occur near zones of adhesion, the future sites of cell division².

The discovery of highly conserved putative GTP-binding domains in SRP54, DP α and two *E. coli* proteins is a further indication of the central role of GTP-binding proteins in intracellular targeting and sorting mechanisms²². □

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Model for signal sequence recognition from amino-acid sequence of 54K subunit of signal recognition particle

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PROTEIN targeting to the endoplasmic reticulum in mammalian cells is catalysed by signal recognition particle (SRP)^{1,2}. Cross-linking experiments have shown that the subunit of relative molecular mass 54,000 (*M*, 54K; SRP54) interacts directly with signal sequences as they emerge from the ribosome^{3,4}. Here we present the sequence of a complementary DNA clone of SRP54 which predicts a protein that contains a putative GTP-binding domain and an unusually methionine-rich domain. The properties of this latter domain suggest that it contains the signal sequence binding site. A previously uncharacterized *Escherichia coli* protein has strong homology to both domains. Closely homologous GTP-binding domains are also found in the α -subunit of the SRP receptor (SR α , docking protein) in the endoplasmic reticulum membrane⁵⁻⁸ and in a second *E. coli* protein, ftsY, which resembles SR α . Recent work has shown that SR α is a GTP-binding protein and that GTP is required for the release of SRP from the signal sequence and the ribosome on targeting to the endoplasmic reticulum membrane⁹. We propose that SRP54 and SR α use GTP in sequential steps of the targeting reaction and that essential features of such a pathway are conserved from bacteria to mammals.

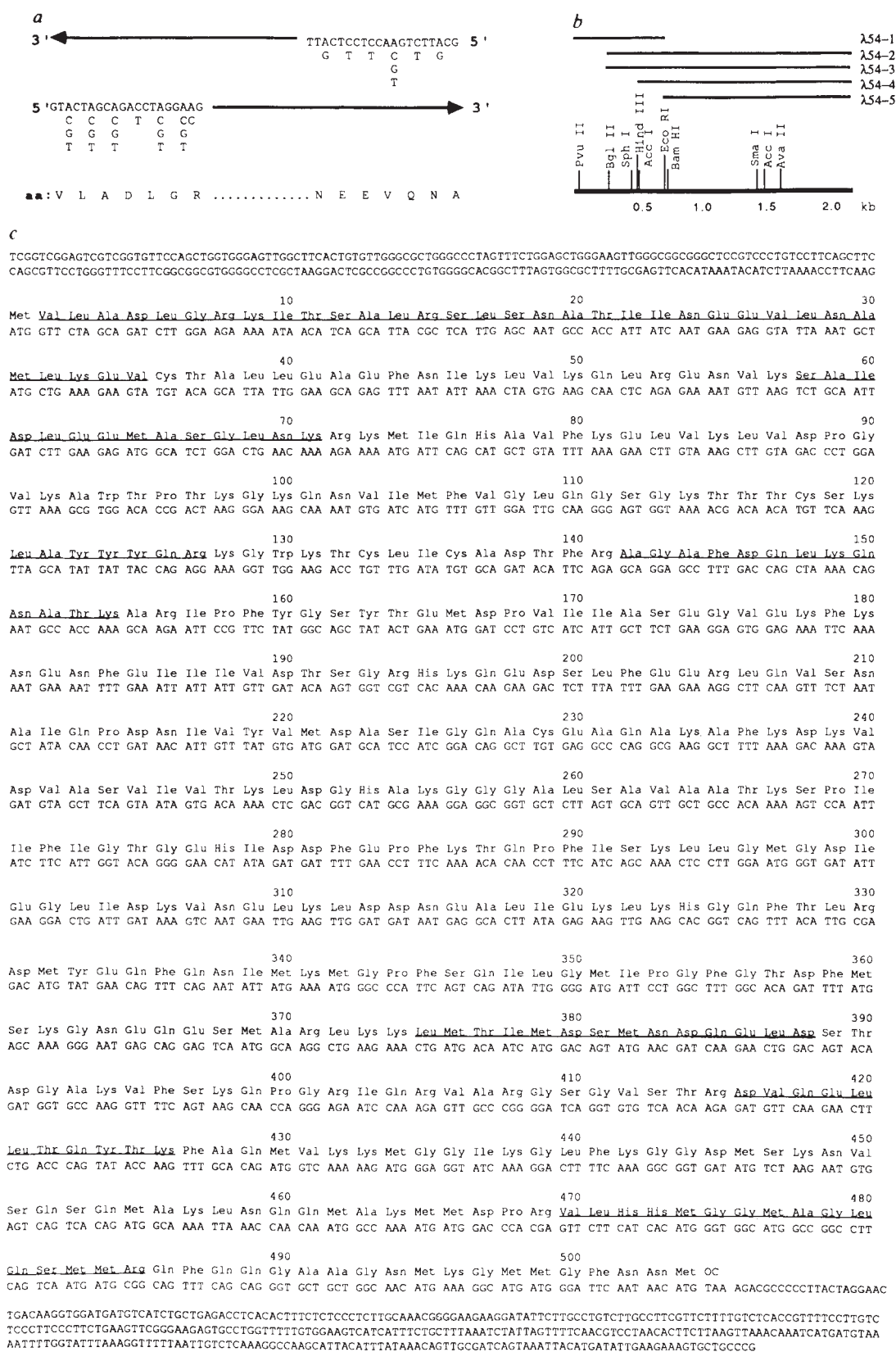
We isolated a cDNA clone encoding SRP54 to understand better the mechanism of signal sequence recognition. SRP was purified to homogeneity from canine pancreas¹ and its protein components separated by preparative SDS-PAGE. SRP54 electroeluted from the gel was sequenced at the N-terminus by Edman degradation. In addition, tryptic fragments were sequenced. From the N-terminal amino-acid sequence, we generated a DNA probe using the polymerase chain reaction¹⁰ (PCR) (Fig. 1A). We used this probe to isolate overlapping cDNA clones from a λ -phage gt10 library (Fig. 1b). Figure 1c shows the cDNA sequence and deduced primary amino-acid sequence of SRP54. Our available protein sequence information was distributed over the entire predicted coding sequence, thus making the identification of the cDNA unambiguous. The N-terminal sequence data match the predicted amino-acid sequence starting immediately after the first AUG found in the cDNA.

Sequence analysis revealed striking homologies of SRP54 to

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FIG. 1. Isolation and sequence of SRP54 murine cDNA clones. *a*, The strategy for the synthesis of an 86-nucleotide fragment of the SRP54 gene using the polymerase chain reaction is shown. Amplification was primed with two degenerate oligonucleotides; one encoded amino acids 2–8, and the other the reverse complement of the DNA encoding amino acids 24–30 of SRP54. All possible codons were represented for each amino acid, except in cases where the genetic code is sixfold degenerate. In those positions the oligonucleotide sequence was based on mammalian codon usage. *b*, Map of cDNA inserts from a λ phage gt10 library. Selected restriction sites are shown. *c*, Nucleotide sequence of the murine SRP54 cDNA and deduced amino-acid sequence. The sequenced portions of the protein are underlined. The cDNA sequence of λ 54-3 contained a point mutation at Tyr 333 (TAT $\rightarrow$ TAA) resulting in a stop codon.

METHODS. Peptide sequencing: SRP54 was purified from purified SRP by SDS-PAGE and electroelution. N-terminal sequence was obtained from one portion (152 pmol) by sequential Edman degradation. The remainder was digested with trypsin and the fragments fractionated by reverse-phase HPLC. Three peaks were collected that contained mixtures of two or three individual peptides, each giving rise to the assignment of more than one amino acid per cycle. Although the starting material was canine SRP and the cDNA clone was from mouse, a perfect match of all peptide sequences was obtained. cDNA cloning: PCR reactions (20 μ l) contained 0.1 μ g of each degenerate oligonucleotide, 5 μ l target cDNA prepared from MDCK cell poly(A)⁺ RNA as described³¹, 0.2 mM dNTPs, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM MgCl₂, 0.1% gelatin, and 0.6 units *Taq* polymerase (Cetus). For amplification of target cDNA we used 40 cycles of denaturation (94 °C, 1 min), annealing (42 °C, 2 min) and extension (72 °C, 3 min). The reaction products were resolved on an 8% polyacrylamide gel. The 86-nucleotide fragment eluted from the gel was sequenced directly³². A λ phage gt10 mouse embryonic cDNA library, prepared according to established methods³³ by Dr C. Hauser, was obtained from Dr M. Frohman. The 86-nucleotide fragment was used as a probe to screen 100,000 λ -phage plaques from the library. One phage that contained a homologous insert (λ 54-1) was obtained. Four additional phage (λ 54-2 to λ 54-5) were identified on screen-



Translated M_r = 55754.97

ing an additional 100,000 plaques using the insert of λ 54-1 as a probe. In the first round of screening, nitrocellulose filters were hybridized for 16 h at 42 °C in 5 × NET (1 × NET = 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM EDTA), 5 × Denhardt's solution, 0.1% SDS, 30% formamide, and 100 μ g ml⁻¹ salmon sperm DNA and washed at 42 °C in 1 × NET, 0.2% SDS. In the second round, 50% formamide was used and the filters were washed in 1 × NET/0.2% SDS at 65 °C. The λ -phage inserts were cloned into mp18 and sequenced³⁴ using Sequenase (US Biochemical). Internal oligonucleotide primers were also used to facilitate sequencing.

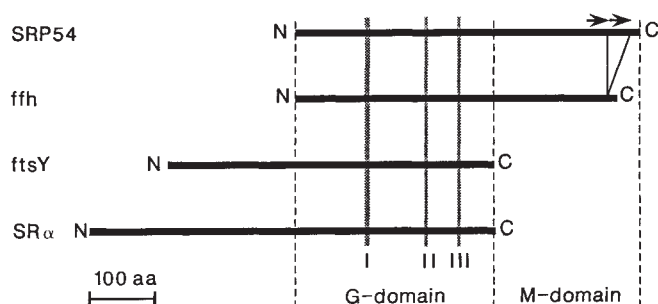


FIG. 2 Schematic alignment of the primary amino-acid sequences of SRP54, ffh, ftsY and SR α . The boundaries defining the homologous G-domains and M-domains are indicated. The sequences of both domains are shown in Figs 3 and 4, respectively. The three boxes comprising the GTP-binding consensus are shaded. Arrows indicate the position of a duplicated 35 amino-acid sequence in the M-domain of SRP54 that is present in ffh only once. The N-terminal regions of ftsY and SR α show no apparent similarity to one another.

three other protein sequences, which also show previously unrecognized homology to one another (see alignments in Figs 2, 3a and 4a). An *E. coli* protein of unknown function is homologous over its entire coding sequence to SRP54 and we designate this previously unnamed gene *ffh* (fifty-four homologue). The *ffh* gene was identified as an open reading frame directly upstream of the *trmD* operon¹¹. We note that the homology to the *ffh* protein begins at an in-frame Met residue 35 amino acids upstream of the originally proposed initiation site, yielding a predicted protein of 50k (ref. 12). Both *ffh* and SRP54 have a GTP-binding domain (G-domain) and a methionine-rich region (M-domain). The G-domain of SRP54 is also homologous to the mammalian SR α (ref. 7) and ftsY, another *E. coli* protein¹³ (Figs 2 and 3a). FtsY is the first gene in an operon encoding proteins involved in cell fission, but the function of ftsY itself is not known. SR α and ftsY are homologous to each other only in their G-domains.

The consensus sequence for a GTP-binding site previously identified in SR α (ref. 9) is contained within the G-domain and is indicated in the alignment with three other known GTP-binding proteins¹⁴ in Fig. 3b. In each case, box III of the four

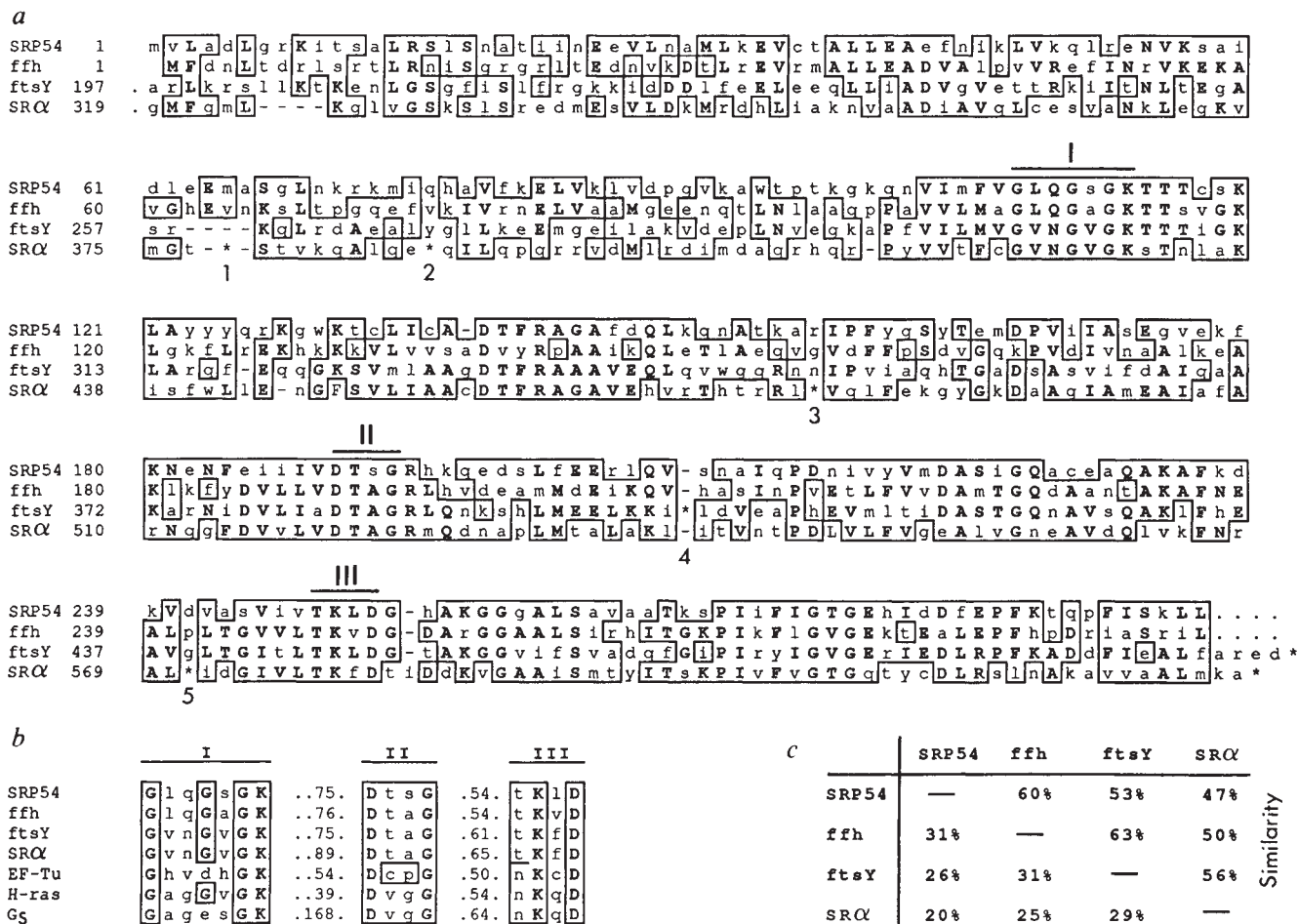


FIG. 3 Sequence comparison of the G-domain of SRP54, ffh, ftsY and SR α . **a**, Alignment of the primary sequences. The position in the primary sequence of the first amino acid on every line is shown. Two or more identical amino acids in one position are indicated by capital letters. Amino acids (one-letter code) of similar chemical properties are boxed, using the following similarity rules: L=I=M=V=F=W, K=R=H, D=E=Q=N, G=A=S, T=V, A=V, F=Y=H=W (ref. 24). Note that some positions are boxed because of two independent pairwise similarities. Gaps are indicated by dashes. Numbers under the alignment indicate the following insertions at the position of the asterisks in the sequences above: (1) FSTVT (2) SLV (3) SALHPPEKHAGPTM (4) VRVMKK (5) ADHSMAQTPLRL. The C terminus of ftsY and SR α is also marked by

asterisks. The positions of the GTP-binding consensus boxes are indicated above the alignment. **b**, Alignment of the GTP-binding consensus boxes of SRP54, ffh, ftsY and SR α with those of three other GTP-binding proteins, *E. coli* EF-Tu, human Ha-ras and rat G_s (ref. 14). The number of amino acids between the boxes is indicated. **c**, Quantification of the homologies in the G-domain of SRP54, ffh, ftsY and SR α . Per cent identity is defined as the ratio of the number of positions containing identical amino acids between any pairwise analysis of sequences aligned as shown in **a** to the total number of amino acids in the shorter sequence. Per cent similarity was computer likewise, except that chemically similar amino acids were counted in addition to identities.

proteins deviates by one amino acid from the consensus (Asn → Thr), yet GTP binding to SR α has been demonstrated experimentally⁹. This deviation from an otherwise conserved motif and the extensive homologies throughout the G-domain (Fig. 3a), which are not found in other GTP-binding proteins, indicate that these proteins represent a distinct subgroup of GTP-binding proteins. We have analysed all the pairwise alignments of the G-domains of the four sequences by comparing amino-acid identity and amino-acid similarity (Fig. 3c). Note that SRP54 and SR α are as similar by either score to one another as they

are to the prokaryotic proteins, indicating the extreme evolutionary conservation of the G-domain.

In mammalian cells the G-domain is found twice in the protein targeting pathway. As SRP and SRP receptor act sequentially, it is conceivable that the binding of SRP to the SRP receptor involves a direct interaction of the two G-domains. By analogy, receptors and ligands in other systems have been found to be evolutionarily related, suggesting that their binding specificity may have evolved from homotypic interactions^{15,16}.

GTP may be used as a switch to provide unidirectionality to

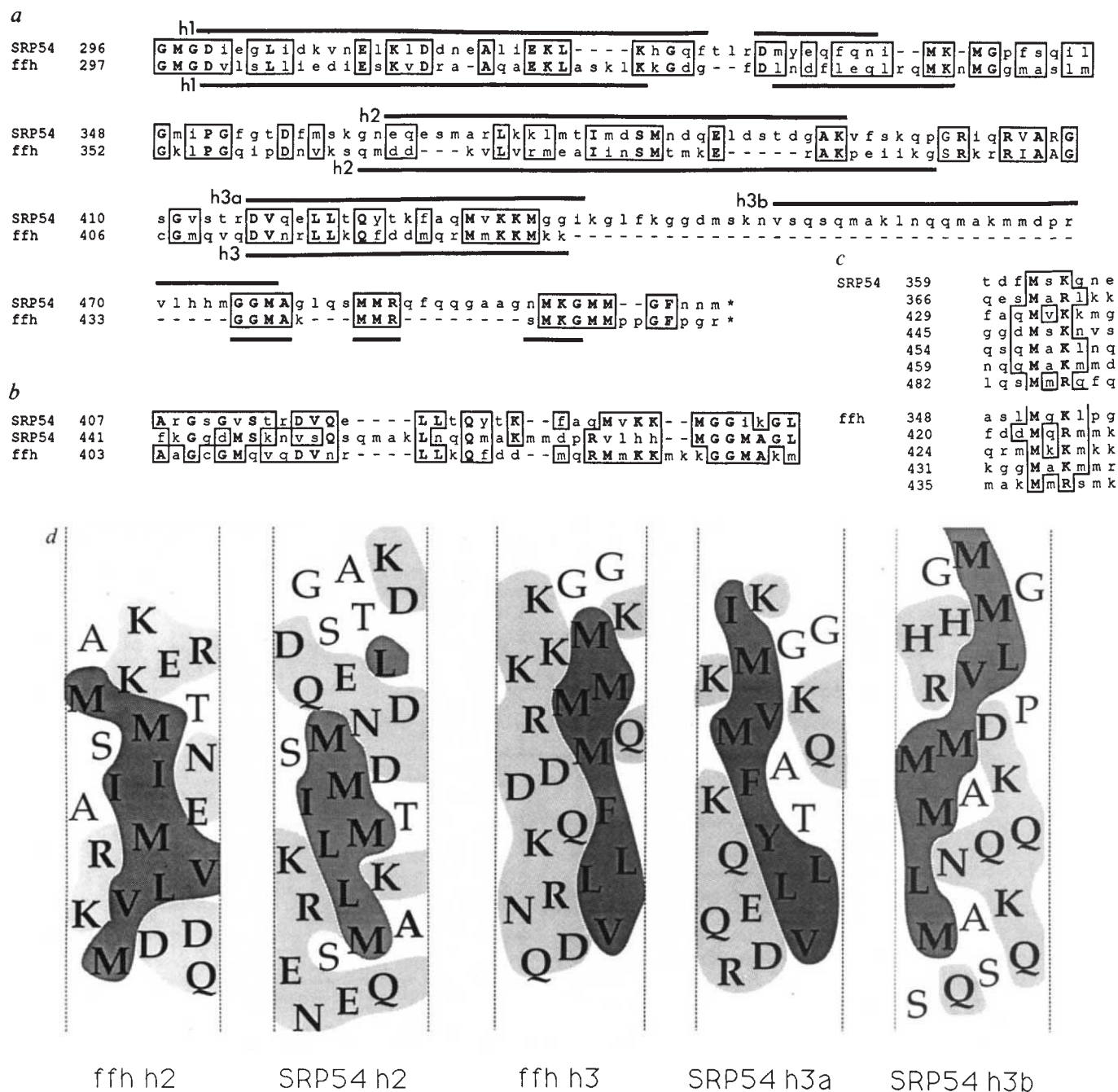


FIG. 4 Sequence analysis of the M-domains of SRP54 and ffh. **a**, The primary sequences of the M-domains of SRP54 and ffh are aligned, and identities and similarities are highlighted as in Fig. 3a. The M-domains of SRP54 and ffh have isoelectric points of 10 and 10.6, respectively. A secondary structure prediction was performed according to established methods²⁰. Regions that are very likely to form α -helices (an average score above 20) are marked above or below the sequences. **b**, Alignment of two repeated sequences in the M-domain of SRP54 with their homologous region in ffh. Helix 3 is part of this sequence in ffh and hence is repeated (helices 3a and 3b) in SRP54.

c, A short pattern matching a loosely defined consensus was found by visual inspection to be multiply repeated in SRP54 and ffh. Note the conservation of the spacing between Met and a basic amino acid. In an α -helical conformation this pattern would result in a strong amphipathicity. **d**, Projection of the postulated amphipathic helices 2 and 3 of SRP54 and ffh. The projections form an α -helix when rolled into a cylinder. Sequences start at the bottom and wind upwards. Strongly hydrophobic amino acids (M, L, I, F, V) are darkly shaded; strongly polar amino acids (E, D, K, R, N, Q, H) are lightly shaded.

individual steps of the targeting reaction. GTP hydrolysis is used in other processes where multiple components must function coordinately¹⁷. For example, eIF-2 monitors the assembly of a complex of at least five separate components before it hydrolyses GTP and protein synthesis is initiated¹⁸. Alternatively, GTP hydrolysis could be used to improve the fidelity of signal recognition. A precedent for such a phenomenon is the kinetic proof-reading of EF-Tu (ref. 19), in which GTP hydrolysis is used as a timing device to improve the fidelity of protein synthesis well beyond the theoretical limits set by the binding energy of the codon-anticodon interaction.

The M-domains of SRP54 and ffh are as well conserved as their G-domains (38% identical, 59% similar; Fig. 4a). These domains are characterized by their unusually high Met contents (11% and 13%, respectively) and a very polar and overall basic character. Many of the Met and basic amino acids are found in a short repeated pattern (Fig. 4c). Secondary structure predictions²⁰ of the M-domain strongly suggest three α -helical regions (indicated in Fig. 4a). Moreover, the predicted boundaries of all three helices coincide almost precisely between SRP54 and ffh. As the primary sequences of SRP54 and ffh are only 38% identical, the alignment of predicted secondary structures between the two proteins strongly supports the validity of the helix assignment. Fourier transform analysis of the hydropathy versus sequence position²¹ indicates that the helices are strongly amphipathic. A projection and comparison of helices 2 and 3 from ffh and helices 2, 3a and 3b from SRP54 is shown in Fig. 4d. Hydrophobic residues including all of the Met residues in the helical regions are clustered exclusively on one face of the helices; patches of strongly polar residues are found on the opposite face. Helices 1 from both ffh and SRP54 are also amphipathic, but do not contain Met residues. SRP54 contains two copies of a 35 amino-acid repeat at the C terminus which is present only once in ffh (see Fig. 2 and Fig. 4b). The last helix (helix 3) in ffh is homologous to the repeated sequence of SRP54 and hence is present twice in SRP54 (helix 3a and 3b).

It is not known which domain of SRP54 contains the signal sequence binding site. If this site were contained within the G-domain, then we could assume that SR α , which contains a homologous domain, might also interact with signal sequences. Signal sequences would then be recognized in a cascade of events and be passed sequentially from SRP54 to SR α to other membrane proteins. Experimental evidence argues against this hypothesis because no cross-linking of signal sequences to SR α has been observed (ref. 22; U. C. Krieg, A. E. Johnson and P.W., manuscript in preparation); this negative result, however, could still be explained by the transient nature of the interaction.

An alternative hypothesis is that signal sequences bind to the M-domain of SRP54. This model is appealing because the unique features of this domain could provide an answer to the puzzle of how signal sequences can bind specifically to a defined pocket on a protein, despite their primary sequence heterogeneity²³. A striking feature of the M-domain is the evolutionarily conserved abundance of Met residues that are predicted to be clustered on one face of two similar α -helices in ffh and three α -helices in SRP54 (Fig. 4d). Met, Leu and Ile residues are comparably hydrophobic and replace one another in phylogenetic comparisons²⁴. Given that Met is usually a rare amino acid, its high abundance in the M-domain is presumably not only of structural, but also of functional importance. A unique structural feature of Met side chains is their flexibility; both Leu and Ile side chains are branched and hence comparatively rigid. We propose that the postulated α -helices of the M-domain could form and/or contribute to a groove on the surface of the protein that constitutes the signal sequence binding site. The spatial arrangement of the helices would be provided by other elements in the domain. The interior of the groove would be composed of hydrophobic amino acids and the abundant Met side chains would project like the flexible bristles of a brush into the groove. Signal sequences could then bind

through hydrophobic interactions and, provided they can conform to the steric constraints imposed by the groove, their widely different primary sequences would be accommodated by the plasticity of the environment. As the groove is not composed only of Met side chains, there may be certain additional constraints at local points that would result in different affinities and possibly allow discrimination among individual sequences.

An analogous situation involving the binding of a diverse population of peptides is found during antigen presentation by major histocompatibility complex molecules. In this case a binding groove as defined by crystallographic analysis²⁵ is flanked by α -helices and contains positionally conserved amino-acid side chains. Many of the invariant amino acids are Tyr that, although unlike Met are inflexible, can undergo both hydrophobic and hydrogen-bonding interactions and hence may allow the protein to accept a variety of ligands.

The functions of both ffh and ftsY are unknown. But, the extended homology of SRP54 and ffh suggests that ffh may function as a signal recognition protein in *E. coli*. The association of ftsY with the inner membrane fraction of *E. coli*²⁶ would be consistent with a possible SRP receptor-like function. Translocation in *E. coli* can occur post-translationally for most proteins²⁷. Ffh and ftsY could be part of this pathway, although so far they have escaped genetic or biochemical detection. Alternatively, they may function in a pathway analogous to SRP-dependent targeting in mammalian cells in which signal recognition is dependent on the context of the ribosome²⁸. Such a pathway might be essential for only a few proteins whose biogenesis requires an obligate link with translation. *E. coli* 4.5S RNA has recently been found to contain a domain structurally homologous to SRP RNA and could also be part of this pathway^{29,30}. Yet in spite of these intriguing homologies, many aspects of the physiology of prokaryotic cells are fundamentally different from eukaryotic cells and therefore seemingly similar components might have additional or distinctly different functions. □

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Transcription factor IIIA induced bending of the *Xenopus* somatic 5S gene promoter

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TRANSCRIPTION factor IIIA (TFIIIA), the canonical zinc-finger protein, is a protein of relative molecular mass 39,000 (39K) that is required for transcription of 5S-ribosomal subunit genes in *Xenopus*¹. It binds in a sequence-specific manner to the internal control region of the 5S gene (see Fig. 1) and facilitates transcription of the gene by RNA polymerase III^{2,3}. It also binds to the 5S gene product to form a 7S ribonucleoprotein particle. In oocytes the 7S particle acts as a storage form of the RNA to be utilized later in development^{4,5}. TFIIIA binds to DNA through its 30 K N-terminal domain, which contains nine zinc-fingers⁶. TFIIIA was the first protein described to have this type of DNA binding motif, but numerous other proteins have now been shown to have zinc-finger domains⁷. A structure for a single zinc-finger from the yeast protein ADRI, was recently proposed based on two-dimensional NMR data (ref. 8), and a similar structure was proposed based on comparison with crystal structures of other metalloproteins⁹. Although models for the interaction of TFIIIA with the 5S-ribosomal gene DNA have been proposed, based on nuclease digestion and methylation interference data¹⁰, little precise structural information is available for TFIIIA and the physical basis for the interaction of zinc-fingers with DNA is not understood. Using both circular permutation and circularization assays we provide convincing biochemical evidence that TFIIIA bends the DNA at the internal promoter of the 5S gene.

Bieker and Roeder determined that the shape of purified TFIIIA is highly asymmetrical and extended, with a Stokes radius of 34 Å¹¹. This indicates that the protein could extend along the 50 base pairs (bp) of the internal control region (ICR). Recently, neutron scattering studies of 7S particles have provided evidence that TFIIIA bound to be 5S RNA is in an extended conformation¹². By contrast, electron microscopy studies suggest that the protein may be able to adopt a compact conformation when bound to the 5S gene¹³. When TFIIIA is bound to closed circular DNA, and the complexes are relaxed with topoisomerase I and then de-proteinized, the linking number of the DNA is reduced¹⁴. This could indicate that TFIIIA unwinds the DNA or that the DNA is bent or wrapped around the protein surface. As circular dichroism measurements indicate no gross alteration of the B-form DNA helix upon protein binding¹⁵, the linking number change most likely reflects bending or wrapping of the DNA on binding of TFIIIA.

The hallmark of bent DNA is its anomalous migration in polyacrylamide gels. Mobility in a polyacrylamide gel matrix also depends on the position of the bend within the fragment¹⁶. Anomalous migration is maximized if the bend is located near the middle of the fragment, and minimized if it is positioned at an end. This behaviour is also seen for protein-induced DNA bending. Several proteins have been shown to exhibit site-specific DNA bending using a circular permutation assay, including the integration host factor¹⁷ and the catabolite activator protein¹⁶ of *Escherichia coli*, and the heat shock transcription factor of *Drosophila*¹⁸.

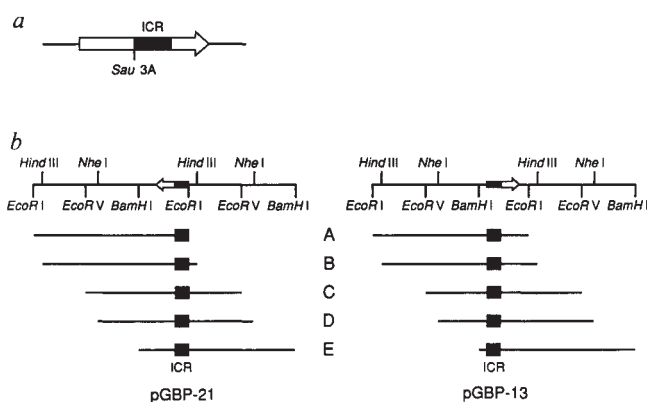


FIG. 1 Structure of DNA molecules used in these experiments. *a*, For both experiments we used a 248 bp *Bam*HI fragment derived from the *X. borealis* somatic-type 5S gene cloned in plasmid pXp-10 (ref. 21). This fragment contains 49 bp of 5' flanking sequence, the 120 bp 5S gene, 69 bp of 3' flanking sequence and *Bam*HI linkers to provide cohesive ends. The position of the ICR is shown. The entire 248 bp fragment was used for the circularization experiments (see Fig. 3). For the permutation assays the 248 bp fragment was cut again with *Sau*3A, which cleaves the fragment just to the 5' side of the ICR. This 150 bp fragment was cloned into the *Bgl*II site of pCY-7, in both orientations. Plasmid pCY-7 is a derivative of pBR322 in which the initial 375 bp *Eco*RI-*Bam*HI segment is present as a tandem repeat²⁷. *b*, The 10 possible circular permutations of the ICR in the newly constructed plasmids pGBP-21 and pGBP-13.

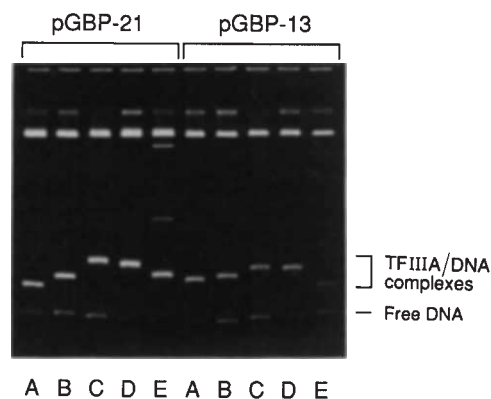


FIG. 2 Polyacrylamide gel electrophoresis (6%) of circularly permuted TFIIIA-DNA complexes.

METHODS. The 10 uniquely positioned fragments, described in Fig. 1*b*, were mixed with TFIIIA for 20 min at 25 °C, under the binding conditions: 20mM Tris, pH 7.5, 70mM KCl, 1.5mM MgCl₂, 2.5mM DTT. Electrophoresis was at 25 °C in 1x Tris-Borate buffer (without EDTA), pH 8.1, and the gel was stained with ethidium bromide. The TFIIIA was prepared as follows: 7S RNP particles were isolated from immature *X. laevis* oocytes using glycerol gradient centrifugation and DEAE-cellulose chromatography²⁸. The purity of the final fraction of TFIIIA was 80–90% as determined by SDS-PAGE. The 7S particles were stored at –80 °C, and treated with 50 µg ml^{–1} RNase A for 10 min at 25 °C, to destroy the 5S RNA before use²³.

The electrophoresis of 550 bp-DNA fragments which contain circular permutations of the ICR from the *Xenopus borealis* somatic-type 5S gene are shown in Fig. 2. Under these electrophoresis conditions no unusual migration is observed for the free DNA. A dramatic effect is seen, however, in the migration of the TFIIIA/DNA complexes, which is highly dependent on the position of the ICR within the fragment. Bending of the DNA by TFIIIA is not affected by the orientation of the ICR; the two plasmids used in this assay contain the ICR in opposite orientations (see Fig. 1*b*), and in both cases the fragments with the most terminal ICR, 21A and 13E, migrate the fastest. The

slowest migrating complexes are 21C and 13D, in which the ICR is centrally located in the DNA fragment.

TFIIIA isolated from *Xenopus* will also bind to the ICR of the sea urchin 5S gene¹⁹. We tested the ability of TFIIIA to bend this DNA in a circular permutation assay. Bending was observed and the migration of the complexes was again dependent upon the position of the ICR within the fragment, as above (data not shown).

Another way of examining the effects of TFIIIA on 5S DNA structure is to monitor the rate of circularization of the fragment containing the ICR in the presence or absence of TFIIIA. If the protein induces a bend it will decrease the distance between the two ends of the molecule and hence increase the probability of ring closure. Such an approach has been used to show site-specific bending by the cyclic-AMP receptor protein²⁰. For our experiments we used the 248 bp *Bam*HI fragment derived from plasmid pXp-10 (ref. 21) (Fig. 1a). Figure 3 shows that a fivefold molar excess of TFIIIA over binding sites stimulates the rate of circularization approximately threefold. Under these conditions, all of the 5S DNA molecules are bound by TFIIIA, as determined by non-denaturing gel electrophoresis (data not shown). Higher concentrations of TFIIIA do not increase the rate of circularization and excess concentration interferes with circularization, presumably by binding non-specifically to DNA termini. TFIIIA at the same five to one ratio has no effects on the rate of circularization of a 258 bp control DNA fragment (derived from *Sau*3A cut pUC-19) lacking the 5S gene ICR

(data not shown). Two possible explanations for the enhancement of circularization rate by TFIIIA are, either the induction of a stable bend in the DNA, or a change in the helix-twist angle favouring intramolecular ligations²². We conclude, based on circular dichroism measurements¹⁵, footprinting experiments^{23,24} and the gel-mobility shift experiments presented here (Fig. 2) that DNA bending seems the most likely explanation for the enhanced circularization rates.

During the course of these studies we noticed that the rate of circularization of the 248 bp *Bam*HI fragment containing the 5S gene was three to four fold higher than that of the 258 bp control fragment. This suggests that the 5S DNA may adopt an unusual structure, perhaps a static bend, that is not detected under our gel electrophoresis conditions (Fig. 2, 'Free DNA'). This discrepancy may reflect differences in the 'bendability' of DNA under the conditions used in the ligations, and warrant further investigation.

The experiments presented here provide convincing biochemical evidence that TFIIIA bends the DNA of the 5S gene when bound to the internal promoter. This has many interesting implications for both the structure and function of TFIIIA and for 5S gene regulation. To our knowledge, TFIIIA is the first zinc-finger protein that has been shown to bend DNA. Since TFIIIA binds to DNA through nine zinc-fingers⁶, and the structural elements of zinc-fingers are very highly conserved⁷, our results suggest that this may be a common property of other zinc-finger proteins. These results, when taken together with recently published electron micrographs of the complex¹³, also suggest that the TFIIIA in the TFIIIA/DNA complex may have a compact, globular shape. This is in direct contrast to the extended conformation that the protein has when free in solution¹¹ or when complexed with the 5S RNA^{12,25}. The possibility that TFIIIA may have more than one active conformational state could explain the ability of the protein to bind both DNA and RNA.

The potential for regulation of the different types of 5S gene expression (that is, oocyte versus somatic) by differential DNA bending or TFIIIA conformational change also exists²⁶. These results will allow development of more detailed models of the interaction of TFIIIA with the 5S gene and the other factors involved in 5S gene expression. □

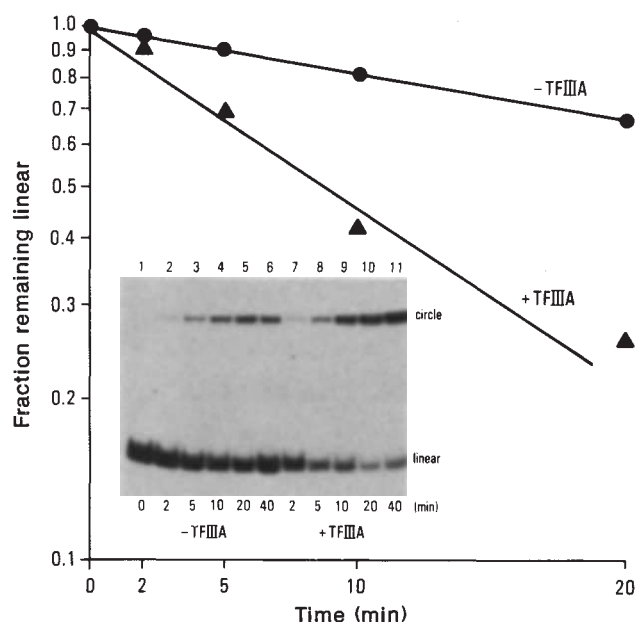


FIG. 3 TFIIIA induced bending of the 5S gene promoter. Inset: autoradiogram of gel showing time course of circularization + or - TFIIIA.

METHODS. The 248 *Bam*HI restriction fragment containing the 5S gene described in Fig. 1a was dephosphorylated with calf alkaline phosphatase and end-labelled with [γ -³²P] ATP and polynucleotide kinase. To ensure that all ends were phosphorylated the reaction was chased with unlabelled ATP (1mM) and additional kinase. The labelled DNA was incubated in the presence or absence of a fivefold molar excess of TFIIIA as 7S RNP (treated with RNase A at 50 μ g ml⁻¹ to destroy 5S RNA) for 20 min at 22 °C in a volume of 25 μ l. The sample was then diluted to 300 μ l with 50mM NaCl, 10mM Tris pH 7.5, 5mM MgCl₂, 1mM ATP and T4 DNA ligase to a final concentration of 0.5 U ml⁻¹. Aliquots (50 μ l) were withdrawn at the indicated times and added to an equal volume of 0.5% SDS, 50mM EDTA, 200 μ g ml⁻¹ proteinase K. After 30 min at 37 °C, the samples were extracted with phenol, ethanol precipitated and subjected to electrophoresis in a 5% polyacrylamide gel. The fraction of linear DNA remaining at each time point was determined by densitometric scanning of the autoradiogram of the gel.

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